THE INTERNAL VASCULARIZATION OF THE BRAIN IN NORMAL AND PROTEIN-DEPRIVED RATS

Nils Conradi

Göteborg 1980

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41544702_0010

THE INTERNAL VASCULARIZATION OF THE BRAIN IN NORMAL AND PROTEIN-DEPRIVED RATS

AKADEMISK AVHANDLING

som, för avläggande av medicine doktorsexamen
vid Göteborgs Universitet, offenligen kommer att försvaras i
Föreläsningssalen, Patologiska institutionen
Sahlgrenska Sjukhuset, Göteborg
kl 13.00 fredagen den 26 september 1980

av

Nils Conradi

Göteborg 1980

ABSTRACT

The Internal Vascularization of the Brain in Normal and Protein-deprived Rats

CONRADI, N. Division of Neuropathology, Department of Pathology, University of Göteborg, S–413 45 Göteborg, Sweden

Thesis based on the following papers:

I. Conradi, N.G. and Sourander, P.: The early internal vascularization of the rat brain. Morphological studies on foetuses of normal and protein-deprived mothers. Acta Neuropathol (Berl) 50: 221–226 (1980).

II. Conradi, N.G., Engvall, J. and Wolff, J.R.: Angioarchitectonics of rat cerebellar cortex during pre- and postnatal development. Acta Neuropathol (Berl) 50: 131–138 (1980).

III. Conradi, N.G., Eins, S. and Wolff, J.R.: Postnatal vascular growth in the neocortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 123–130 (1979).

IV. Conradi, N.G., Eins, S. and Wolff, J.R.: Postnatal vascular growth in the cerebellar cortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 131–137 (1979).

The formation and branching of stem vessels in the embryonic brain and the cerebellum and the growth of capillaries in the cerebral and cerebellar cortex have been examined. There is a temporal and spatial correlation between the ingrowth of the first vessels and the appearance of neurons and glioblasts in the embryonic brain. The formation of stem vessels parallels the formation of fissures in the cerebellum and, thus, does not merely follow the expansion of the cerebellar surface but appears to be controlled by factors in the cerebellum. Stem vessels terminate earlier in deep than in superficial levels of the brain. The level of termination of stem vessels appears to be an indication of their time of formation. Branches of stem vessels first give rise to a deep and an superficial plexus in the ventricular zone and, later, to a plexus in the subventricular and intermediate zones. The termination of some stem vessels in the cerebellar cortex shows a close spatial relation to the Purkinje cells. The latest stem vessels in the cerebellum may form and terminate in relation to the differentiation of neurons. The formation and growth of capillaries show distinct temporal and spatial relations to the differentiation of neurons, notably synaptogenesis and increased oxidative metabolism. This is nicely illustrated by the formation of a capillary plexus in the Purkinje cell layer during the early postnatal development in the rat. Part of the studies was performed on protein-deprived rats. Protein deprivation was achieved by feeding female rats a low protein diet from two weeks before mating, during lactation and gestation and weaning their offspring on the same diet. Protein deprivation appears not to influence the formation and termination of stem vessels in the embryonic brain during the period embryonic day 12 – 15. The rapid growth of capillaries in the neo-cortex is delayed by some 3 days and the normal increase in the specific length of vessels in neocortical Lamina IV is delayed by at least 10 days in protein deprived rats. The specific length of vessels was increased in the Purkinje cell layer in 7 and 15 day old rats and reduced in the granular and molecular layers in 10 – 20 day old protein deprived rats compared with controls. The findings on the growth of capillaries in protein-deprived rats correlate well to earlier findings on impaired differentiation of neurons in PCM. Several studies have demonstrated an altered development of neurons and glia in PCM rats, the vessels thus cannot compensate for the protein deprivation. There may, however, be a reduced ability of the vessels to respond under other pathological conditions such as hypoxia or hypoglycemia.

Key words: rat – brain – blood vessels – growth – prenatal – postnatal – protein deprivation.

ISBN 91–7222–323–5

Division of Neuropathology
Department of Pathology University of Göteborg,
Göteborg Sweden

THE INTERNAL VASCULARIZATION OF THE BRAIN IN NORMAL AND PROTEIN-DEPRIVED RATS

Nils Conradi

Göteborg 1980

ABSTRACT

The Internal Vascularization of the Brain in Normal and Protein-deprived Rats

CONRADI, N. Division of Neuropathology, Department of Pathology, University of Göteborg, S–413 45 Göteborg, Sweden

Thesis based on the following papers:

I. Conradi, N.G. and Sourander, P.: The early internal vascularization of the rat brain. Morphological studies on foetuses of normal and protein-deprived mothers. Acta Neuropathol (Berl) 50: 221–226 (1980).

II. Conradi, N.G., Engvall, J. and Wolff, J.R.: Angioarchitectonics of rat cerebellar cortex during pre- and postnatal development. Acta Neuropathol (Berl) 50: 131–138 (1980).

III. Conradi, N.G., Eins, S. and Wolff, J.R.: Postnatal vascular growth in the neocortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 123–130 (1979).

IV. Conradi, N.G., Eins, S. and Wolff, J.R.: Postnatal vascular growth in the cerebellar cortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 131–137 (1979).

The formation and branching of stem vessels in the embryonic brain and the cerebellum and the growth of capillaries in the cerebral and cerebellar cortex have been examined. There is a temporal and spatial correlation between the ingrowth of the first vessels and the appearance of neurons and glioblasts in the embryonic brain. The formation of stem vessels parallels the formation of fissures in the cerebellum and, thus, does not merely follow the expansion of the cerebellar surface but appears to be controlled by factors in the cerebellum. Stem vessels terminate earlier in deep than in superficial levels of the brain. The level of termination of stem vessels appears to be an indication of their time of formation. Branches of stem vessels first give rise to a deep and an superficial plexus in the ventricular zone and, later, to a plexus in the subventricular and intermediate zones. The termination of some stem vessels in the cerebellar cortex shows a close spatial relation to the Purkinje cells. The latest stem vessels in the cerebellum may form and terminate in relation to the differentiation of neurons. The formation and growth of capillaries show distinct temporal and spatial relations to the differentiation of neurons, notably synaptogenesis and increased oxidative metabolism. This is nicely illustrated by the formation of a capillary plexus in the Purkinje cell layer during the early postnatal development in the rat. Part of the studies was performed on protein-deprived rats. Protein deprivation was achieved by feeding female rats a low protein diet from two weeks before mating, during lactation and gestation and weaning their offspring on the same diet. Protein deprivation appears not to influence the formation and termination of stem vessels in the embryonic brain during the period embryonic day 12 – 15. The rapid growth of capillaries in the neo-cortex is delayed by some 3 days and the normal increase in the specific length of vessels in neocortical Lamina IV is delayed by at least 10 days in protein deprived rats. The specific length of vessels was increased in the Purkinje cell layer in 7 and 15 day old rats and reduced in the granular and molecular layers in 10 – 20 day old protein deprived rats compared with controls. The findings on the growth of capillaries in protein-deprived rats correlate well to earlier findings on impaired differentiation of neurons in PCM. Several studies have demonstrated an altered development of neurons and glia in PCM rats, the vessels thus cannot compensate for the protein deprivation. There may, however, be a reduced ability of the vessels to respond under other pathological conditions such as hypoxia or hypoglycemia.

Key words: rat – brain – blood vessels – growth – prenatal – postnatal – protein deprivation.

ISBN 91–7222–323–5

UNIV. LEIDEN BIBL.

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. CONRADI, N.G. and SOURANDER, P.: The early internal vascularization of the rat brain. Morphological studies on foetuses of normal and protein-deprived mothers. Acta Neuropathol (Berl) 50: 221–226 (1980).

II. CONRADI, N.G., ENGVALL, J. and WOLFF, J.R.: Angioarchitectonics of rat cerebellar cortex during pre- and postnatal development. Acta Neuropathol (Berl) 50: 131–138 (1980).

III. CONRADI, N.G., EINS, S. and WOLFF, J.R.: Postnatal vascular growth in the neocortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 123–130 (1979).

IV. CONRADI, N.G., EINS, S. and WOLFF, J.R.: Postnatal vascular growth in the cerebellar cortex of normal and protein-deprived rats. Morphometric studies. Acta Neuropathol (Berl) 47: 131–137 (1979).

CONTENTS

GENERAL INTRODUCTION 5

REVIEW OF LITERATURE 6

The internal vascularization of the brain 6
The morphology of growing vessels in the brain 6
Formation and branching of stem vessels 7
Growth of capillaries in the brain in correlation with other variables 8
Growth of capillaries in the brain under pathological conditions 9

The morphogenesis of the brain 10
The embryonic brain 10
Formation and differentiation of neurons in the cerebral cortex 11
Formation and differentiation of neurons in the cerebellar cortex 12
Development of oxidate metabolism 13
Some aspects on the development of neuroglia 14
Hormonal influences on the morphogenesis of the brain 14

Development of the brain in protein-calorie malnutrition 14
Formation and migration of neurons in the cerebral cortex 14
Differentiation of neurons in the cerebral cortex 15
Formation and migration of neurons in the cerebellar cortex 18
Differentiation of neurons in the cerebellar cortex 19
Glial cells 19
Blood vessels 19

AIMS OF THE INVESTIGATION 20

MATERIAL AND METHODS 20

Animals and Diets 20
Morphological studies on vascular development in the embryonic brain 23
Morphological studies on vascular development in the cerebellum 23
Morphometrical studies on the vascular development in cerebral and cerebellar cortex 24

RESULTS 26

Formation and branching of stem vessels in the embryonic brain 26
Formation and branching of stem vessels in the cerebellum 28
Growth of capillaries in the cerebral cortex of normal rats 30
Growth of capillaries in the cerebral cortex of protein-deprived rats 32
Growth of capillaries in the cerebellar cortext of normal rats 33
Growth of capillaries in the cerebellar cortex of protein-deprived rats 36

DISCUSSION 38

Formation and branching of stem vessels 38
Growth of capillaries in the brain 41
The control of the growth of capillaries in the brain 43
The significance of the growth of vessels in the brain in protein deprivation 44

SUMMARY AND CONCLUSIONS 46

ACKNOWLEDGEMENTS 48

REFERENCES 49

GENERAL INTRODUCTION

Deficiency of one or more nutriments in the growing individual may result in stunted growth and development. Such stunting although uncommon may be seen as *e.g.* intra-uterine growth restriction, or "failure to thrive" in the industrialized countries (*cf.* Morgane *et al.* 1978). Malnutrition is a severe problem in the developing countries. According to Bengoa (1974) 100 million children were suffering from malnutrition in the early seventies. Malnutrition may be due to a deficiency of protein (kwashiorkor), a deficiency of calories (marasmus), or various combinations of both (marasmic-kwashiorkor). These types of malnutrition are generally referred to as protein-calorie malnutrition (PCM) (*cf.* Viteri *et al.* 1964, Latham 1974). Research on the effects of PCM on the developing human being is complicated by its occurrence in association with chronic infections, the possible influence of other socio-economic factors and the lack of correct information on the age of the individual (*cf.* Scrimshaw *et al.* 1968, DeLicardie & Cravioto 1974). Several reports, however, indicate that PCM affects the development of the human brain not only in terms of its weight and size but also with regard to the intellectual capacity (*cf.* Brown 1966, Engsner *et al.* 1974a, b, DeLicardie & Cravioto 1974).

The difficulties associated with research on human PCM have led to the increased use of animal models. Some experimental studies on the effects of PCM on the developing individual have been concerned mainly with final effects in the adult animal. These studies may increase our understanding of morphological, biochemical or functional changes induced by PCM during development. Experimental studies on the effects of PCM during development may also further our understanding of different developmental periods and the factors controlling normal development. For this purpose, it is necessary to compare at short time intervals the development of malnourished animals with that of normal controls.

Various experimental models for PCM have been described in the literature. They fall into four categories: 1. increased litter size, 2. restricted suckling time, 3. restricted food-intake and 4. protein deprivation. Categories 1 and 2 may be used for studies on the effects of PCM during development after birth. Maternal PCM will decrease the amount of milk produced and, thereby, available to the offspring. The mother rats and their offspring can thus be subjected to the nutritional insult during both gestation and lactation by categories 3 and 4 (*cf.* Shoemaker & Bloom 1977).

With regard to the effects of experimental PCM on the development of the brain it would clearly be an oversimplification to regard them as reduced building only due to a reduction of available building blocks. Human and experimental PCM affect the endocrine glands, liver, intestines, blood, placenta etc. with possible secondary consequences for the development of the brain (*cf.* Viteri *et al.* 1964, Nowak & Munro 1977). The various experimental models affect the social environment in different ways *e.g.* in respect of mother-pup interaction (*cf.* Plant 1970, Crnic 1976, Punch & Ivinskis 1977). The cells in the brain affect the growth and differentiation of each other and alterations during an early stage will presumably influence the subsequent development (*cf.* Jacobson 1978).

Since amino acids and other nutriments are transported to the brain by the blood vessels the growth and differentiation of the vessels of the brain must be of significance for its growth and development. However, this problem has not been investigated in previous studies on experimental PCM. Transport of amino acids from the blood to the brain has received some interest but the transport

of amino acids is also influenced by their concentration in the blood (*cf.* Nowak & Munro 1977). The development of cell populations can be divided into periods of cell formation, "physiological" cell death, cell migration and cellular differentiation. The vessels of the brain increase in length and number of branches by the mitotic division and elongation of endothelial cells (Klossovskii 1963, Bär & Wolff 1973, Bär 1980). Cytological differentiation is of importance for the development and maintenance of the blood-brain barrier including the transcellular transport systems for *e.g.* amino acids (*cf.* Bradbury 1979). The growth and differentiation of the vessels appear to be influenced by both neurons and glial cells (see below). It is therefore necessary to compare findings on the development of vessels with findings on the development of neurons and glial cells.

REVIEW OF LITERATURE

THE INTERNAL VASCULARIZATION OF THE BRAIN

Morphology of the growing vessels in the brain

The first vessels in the brain are sinusoids which grow with patent lumina (Bär & Wolff 1972). The endothelial cells of these vessels are variably thick (Caley & Maxwell 1970, Bär & Wolff 1972). Their cytoplasm contains large vesicles which appear to be formed by the confluence of cytoplasmic flaps (Donahue 1964). The external surface of sinusoids is not covered by a basement membrane and contacts with other cells are sparse (Caley & Maxwell 1970, Bär & Wolff 1972). The endothelial lining of sinusoids does not act as an efficient barrier to macromolecules transported in the blood (Delorme *et al.* 1970).

Capillary branches of vessels in the brain are formed by so called sprouting (Klossovskii 1963). A sprout is formed when one endothelial cell becomes directed outwards from its vessel of origin. Further endothelial cells are added to the sprout by the mitotic division of the endothelial cells and the sprout will consist of a solid cord of endothelial cells. That part of the sprout which is most distant from the vessel of origin sends off numerous cytoplasmic processes and when this part has contacted another vessel, the lumen opens. The lumina of some segments of the capillaries in the brain are lined by a single endothelial cell without junctions (Wolff 1964). These so called seam-less endothelial cells are probably the result of the process of opening of the sprouts which probably takes place by confluence of intra-cellular vesicles (Wolff & Bär 1972).

Contacts between adjacent endothelial cells in brain vessels are of the tight type from early stages of development (Caley & Maxwell 1970, Bär & Wolff 1972). Processes of astrocytes cover an increasing extent of the external surface of the capillaries in the brain, and cover almost 100 per cent of the surface of capillaries in 20 day-old rats (Wolff & Bär 1976). No, or only a few, new capillary branches are formed after this age (Bär & Wolff 1973). Basement membranes are formed concommitant with the formation of perivascular processes of astrocytes (Bär & Wolff 1972). The development of the blood brain barrier is better correlated to the formation of basement membranes and perivascular processes of astrocytes than to any change in the morphology of the endothelial cells (*cf.* Bradbury 1979).

Differences in patterns of growth exist between stem vessels and capillaries in the brain (*cf.* Wolff *et al.* 1975, Bär 1980) and they will therefore be discussed separately.

Formation and branching of stem vessels

During early development mesenchymal cells form islands of "angioblasts" which give rise to sacs lined by endothelial cells and containing immature blood cells. The ability of mesenchyme to form angioblasts appears with few exceptions to be lost after the early embryonic period. The endothelial cell sacs fuse to form immature "arterial" and "venous" networks, which form new vessels and gradually surround different tissues and organs. They are successively remodelled into arteries and veins. The first step leading to the establishment of circulation in most organs, including the brain, is thus the formation of an epiparenchymal vascular plexus *i.e.* an external vascularization (*cf.* Kaplan & Ford 1966, Wolff *et al.* 1975).

Most authors agree that the earliest vascular network in the brain consists of straight vessels running from the epiparenchymal vascular plexus and their branches which form a plexus close to the ventricles. Which of the straight vessels are arterial and which venous has not been determined; therefore the term stem vessel is used. Tilney and Casamajor (1917) regarded the earliest vessels in the rat telencephalon to be formed by the fusion of "endo-parenchymal" angioblastic islands and that the contact between the "ento-neural" plexus and the "peri-neural" plexus via straight stem vessels was formed later by confluence. Strong (1964) from his results on ink-injected embryonic rabbits agreed with this opinion. Ingrowth of stem vessels preceding the formation of the first endo-parenchymal vascular plexus in the central nervous system of the embryonic chick was described by Williams (1937). The findings of Feeney and Watterson (1946) in the chick spinal cord indicate the same relation between the formation of stem vessels and the endo-parenchymal plexus. Recent studies on the embryonic rat telencephalon have demonstrated the ingrowth of sinusoidal stem vessels, prior to the formation of the plexus of vessels in the ventricular zone (Bär & Wolff 1972, Wolff *et al.* 1975).

There has been some controversy as to whether the internal vascularization of the brain results from the elongation of vascular hair-pin loops or the successive formation of arterio-venous and net capillaries between unpaired stem vessels. The former view appears to have been proposed by Schöbl (1898) and Sterzi (1904) (*cit.* by Wislocki & Campbell 1937) from results on amphibians and reptiles and was supported by Lewis (1957) from results on rabbit embryos. Hair-pin loops have also been described in the brain of the opposum by Wislocki and Campbell (1937) and by Scharrer (1939, 1944) and in the brain of the cat by Klossovskii (1963). Most studies have not, however, recorded hair-pin loops of vessels in the brain of vertebrates (Williams 1937, Craigie 1938, Feeney & Watterson 1946, Lierse 1963, Strong 1964, Duckett 1971, Bär & Wolff 1972, Wolff *et al.* 1975).

Tilney and Casamajor (1917) stated that the earliest "ento-neural" plexus in the brain was never more distant from the ventricular system than the thickness of 2 – 3 cells. Williams (1937) described three zones in the hindbrain of the embryonic chick – a germinal cell zone bordering the ventricle, a zone of epithelial cell nuclei and a non-nucleated marginal velum – and regarded the first plexus of vessels to be located just under the zone of the nuclei of epithelial cells. Strong (1964) referred to the earliest plexus as being subependymal although at this stage no ependyme is present (*cf.* Boulder Committee 1970).

The angioarchitectonics of the embryonic brain after the establishment of the first endo-parenchymal plexus have been most thoroughly described by Williams (1937, chick), Feeney & Watterson (1946, chick) and Strong (1964, rabbit). By the successive formation of stem vessels and "tiers"

of vessel branches, the vascular plexus gradually approaches the outer surface. After the formation of multiple vascular tiers, Strong (1964) described a period of both formation and degeneration of vessels. During this period some of the early formed stem vessels degenerate and the peri-ventricular vessels will partly become disconnected from the epi-parenchymal plexus (Strong 1964, Wolff *et al.* 1975).

Following the successive deposition of neurons in the cerebral cortex of the rat and the concomitant expansion of the brain surface, new sets of stem vessels are formed (Wolff 1978, Bär 1980). Stem vessels which are formed early will terminate in deep regions and stem vessels formed later will terminate in successively more superficial levels (Wolff 1978, Bär 1980).

Very few studies on the development of stem vessels in the cerebellum have been reported (Feeney & Watterson 1946, Klossovskii 1963). Feeney and Watterson (1946) noted that several stem vessels entered the cerebellar cortex at the early shallow fissures but did not discuss this finding. Aby (1899) described stem vessels terminating in three different levels of the cerebellum of the adult cat; the molecular layer, the granular layer and the white matter. Uchimura (1929) paid special attention to stem vessels which turned and followed the Purkinje cell layer for some length before terminating. A recent study (Lange & Halata 1980) has indicated that these vessels are venules.

Growth of capillaries in the brain in correlation with other variables

The vascular density in any one region of the adult brain is the result of vascular growth during development and thus correlations between vascular growth and other variables such as activity of oxidative enzymes and density of synapses can be made from findings on the adult animal also. Earlier suggested correlations have been based on morphological comparison and morphometrical quantitation of differences in vascularity. Many variables alter rapidly during the development of the brain and a positive correlation between any two variables may easily be found during this period. Gyllensten (1959a) listed a number of changes in the visual cortex of mice taking place during the period of rapid vascular growth.

Early studies suggested that regional differences in vascularity were related to differences in metabolic or functional activity (Aby 1899). The first quantitation of vessels per volume in the brain was made by Craigie (1920). The morphometrical analysis used in this and other studies (see *e.g.* Craigie 1921, 1924, 1925, 1938, Cobb & Talbott 1927, Alsén & Petrén 1939, Gyllensten 1959a, b) is based on measurements of the length of the segments of vessels in areas of thick sections, *i.e.* in a certain volume of tissue. Due to the fact that the measurement is performed on the projection of the vessel and not on the vessel itself, the values on vascularity may be questioned. The method does, however, allow approximate comparisons between different regions. Other studies have used the number of vascular profiles per area of sections (*e.g.* Eayrs 1954).

The blood vessels of the brain can be regarded as a thin, randomly orientated cylinder enclosed in a volume of tissue. Thin sections through the volume will cut the cylinder in several places which may vary in number, total circumference and total area per area of the sections. The number of these places per area (N/mm^2) is proportional to the length of the cylinder per volume of the tissue (mm/mm^3, Specific length Lv). The total circumference of the cuts through the cylinder per area (mm/mm^2) is proportional to the surface area of the cylinder per volume of the tissue (mm^2/mm^3, Specific surface, Sv). The total area of the cuts through the cylinder per area (mm^2/mm^2) is proportional to the volume of the cylinder per volume of the tissue (%; volume fraction, Vv)

(*cf.* Elias *et al.* 1971). This morphometrical approach has been used in several recent studies on the vessels of the brain (*cf.* Bär 1980). Due to the principle of vascular growth by sprouting the number of branching points of vessels per volume of the tissue may also be used as a measure of vascular growth (Bär & Wolff 1973).

From his findings on rat brain Craigie (*cf.* Craigie 1938) suggested a correlation between 1. higher length of vessels in Lamina IV of the neocortex of the rat and the termination of the thalamo-cortical fibres in this Lamina and 2. the increase in the length of vessels in the motor cortex of the rat during development and signs of motor activity. In 1937 Dunning and Wolff, demonstrated a correlation between the density of vessels and the amount of dendrites and terminal axonal arborizations in different brain regions of the adult cat. Also in the brain of the cat Campbell (1937) correlated regional differences in vascularity and "oxidase" activity. Scharrer (1945) drew attention to the correlation between the density of mitochondria in the neurons and neuropil and the density of vessels in the brain. The rapid increase in the vascularity of the cerebral cortex takes place during the period of increased oxidative metabolism and formation of synapses, accompanied by a decrease in the water content, glial proliferation and onset of functional activity (*cf.* Gyllensten 1959a, Bär & Wolff 1973, Wolff 1978, Bär 1980).

Growth of capillaries in the brain under pathological conditions

Morphometrical variables which describe the vascularity of the brain are expressed as length etc. per volume of tissue. Thus, the problem emerges whether to make corrections for an alteration of the total volume of the region during development or caused by a pathological condition. Craigie (1924) suggested a method of correction for the shrinkage which takes place during preparation of the tissue. This method is based on the assumption that the shrinkage is similar in all directions. This assumption is questionable for the changes in volume of the cerebral and cerebellar cortex which take place during development or are caused by a pathological condition since the cortex of both regions is laminated and thus not similar in all directions. The growth of capillaries appears to be at least partially related to or even controlled by factors within the nervous tissue itself. It thus seems most appropriate to describe the vascularity in relation to the volume of the tissue. Moreover other structural components of the nervous tissue have been described by their number etc. per volume of tissue thus allowing direct comparison.

The blood vessels of the brain may elongate both by multiplication and by elongation of endothelial cells. A mean length of endothelial cells can be estimated from the mean length of the nuclei of the endothelial cells and the relative frequencies of cross-sectioned vessels without and with such nuclei (Bär & Wolff 1973). The mean length of endothelial cells may be used for the study of a possible mechanism compensating for an absolute or relative decrease in the number of endothelial cells. Such mechanisms may also be surveyed by the quantitation of the specific surface or the volume fraction of the lumina of the vessels. Recent findings on the length of the contacts between endothelial cells have shown that the vessels of the brain may increase in length by a decrease in the length of these contacts (*cf.* Bär 1980).

Eayrs (1954) described a decreased density of vessels in the cerebral cortex of young cretinous rats discussing this finding as being either a primary pathogenetic mechanism or secondary to an altered development of the cortex. In the light of present knowledge it seems reasonable to regard impaired cortical development as primary to the decreased growth of vessels. The increased size of

vascular fragments in sections of the cretinous brains was discussed by Eayrs (1954) as dependent on a distension of vessels by venous congestion or as signifying an increase in the diameter of vessels compensating for the decreased density of vessels in the brain.

Gyllensten (1959a) found an increased length of vessels per volume in the visual cortex of 20 day-old mice bred in darkness from birth. The length of vessels per volume in the visual cortex was unaltered in 30 day-old mice subjected to the same condition. After compensating for decreased cortical volume estimated from data on the amount of neuropil in sections the "absolute" length of vessels was unaltered at 20 days but decreased at 30 days of age. Gyllensten (1959a) thus regarded the changes in the cortex itself to be primary to the alteration of the vascularity. Later studies corroborate the findings of altered neuronal differentiation in sensory deprivation (see below). The relative vascular over-growth in 20 day-old mice is remarkable but may be explained as the result of sensory deprivation resulting in decreased growth of the cortex but not affecting the intracortical factors stimulating the growth of the vessels (see below). Gyllensten (1959b) in another series of experiment investigated the vascularity of the visual cortex of mice bred for different periods after birth in 90 – 100 per cent oxygen. There was a decreased growth of vessels in the visual cortex during the stay in the high oxygen tension. An increased length of vessels per volume was seen in mice 35 days of age after a stay in high oxygen tension for 20 days and in air for 15 days. These findings seem to illustrate the relation between vascular growth and the aerobic metabolism but the alterations may also result from toxic effects of oxygen on the retina or the visual cortex (*cf.* Gyllensten 1959b).

Hypoxia at normal athmospheric pressure during days 4 – 14 postnatally resulted in an increased proliferation of the endothelial cells accompanied by an increase in the specific length and diameter of lumina of vessels (Bär 1980). The number of branching points was not altered. The increase in the specific length of vessels after hypoxia during days 14 – 54 postnatally was merely due to a severely reduced volume of the cortex. Hypoxia during days 30 70 postnatally resulted in an increased specific length and diameter of the lumina of vessels, while during days 80 – 120 postnatally it only resulted in a dilatation of vessels. The results illustrate the variable sensitivity of the cortex to prolonged hypoxia during different developmental stages (Bär 1980). The findings also seem to illustrate the relation between oxidative metabolism and the growth of vessels in the brain. Hypoxia, however, affects also the relative frequencies of type I and type II synapses in the visual cortex indicating an negative effect on the differentiation of neurons (Bär 1977). Vascular adaptation to the hypoxic state is insufficient or to slow to compensate for the hypoxia during early developmental stages. This is most definitely so during the period of the most rapid increase in the activity of the oxidative metabolism *i.e.* after 14 days postnatally in the rat (Bär 1980).

THE MORPHOGENESIS OF THE BRAIN

The embryonic brain

The neural tube, after primary neural induction (*cf.* Saxén & Toivonen 1962), folding and closing, consists of a pseudostratified layer of ventricular cells forming the ventricular zone (Boulder Committee 1970). The outer surface of the neural tube is covered by a basement membrane; the inner surface, facing the ventricular system, is not. The ventricular cells are closely attached to each

other by terminal bars at the inner "ventricular" surface. A thin radial cytoplasmic process extends to the outer "pial" surface. The nuclei of the ventricular cells move in the cytoplasm of the radial processes towards the outer surface, then return to the inner surface and undergo mitosis (Sauer 1935a, b, 1936, Sauer & Chittenden 1959, Sauer & Walker 1959, Sidman *et al.* 1959, Fujita 1963). The DNA synthesis takes place during the period of nuclear migration (Sauer & Chittenden 1959, Sauer & Walker 1959). Berry and Rogers (1965), in contrast to Sauer (1935a, b, 1936) found that the ventricular cells did not loose their attachment to the pial surface even during mitotic division of the nucleus. The cleavage plane of the mitotic nucleus of the ventricular cells is at first perpendicular to the ventricular surface and both daughter cells appear to reenter the mitotic cycle. Later some mitotic spindles will have a cleavage plane which is more or less parallel to the ventricular surface. The outer daughter cell may then loose its contacts with other ventricular cells at the inner surface and migrate towards the outer surface (Martin & Langman 1965, Martin 1967). Recent findings indicate that ventricular cells with a perpendicular cleavage plane loose the outer contact during mitosis while these with a parallel cleavage plane may retain it (Hinds & Ruffet 1971, Rickmann & Wolff 1976). Immature neurons that are formed in the ventricular zone are post-mitotic whereas glioblasts and precursors or certain types of neurons *e.g.* the cells of the external granular layer of the cerebellum may migrate and later undergo new mitotic divisions (*cf.* Altman 1972a, Jacobson 1978).

The next zone to be formed in the wall of the embryonic brain is the marginal zone which is located outside the ventricular zone (*cf.* Boulder Committee 1970). The marginal zone from the time of its formation consists of immature neurons, glioblasts and nerve fibres (Raedler & Sievers 1976, Rickmann & Wolff 1976). The next zone to be formed, the intermediate zone, is located between the marginal and ventricular zones and consists of immature migrating neurons. This zone may either remain simple or get more complex due to secondary re-arrangements of cells (*cf.* Levi-Montalcini' 1964, Källén 1965). The subventricular zone is formed after the intermediate zone and is located next to the ventricular zone. The cells in the subventricular zone may undergo mitotic division and probably give rise to macroglial cells and certain classes of small cortical interneurons (*cf.* Boulder Committee 1970).

Formation and differentiation of neurons in the cerebral cortex

Most neurons of the cerebral neo-cortex reach their final position at or shortly after birth in the rat (Berry *et al.* 1964). The neurons of the neo-cortex are generally formed in the ventricular zone between embryonic day 16 and 21 (Berry *et al.* 1964). Their migration to the cortical plate is probably guided by radial glial fibres most of which extend from the inner to the outer surface of the embryonic brain (Rakic 1972). The vertical arrangement of neurons in the adult cortex, which may correspond to functional columns (Mountcastle 1957, *cf.* Chow & Leiman 1971) has been suggested to result from the mode of migration (Rakic 1974).

Migrating neurons are successively deposited at the outer surface of the cortical plate leading to the so called inside-out layering of cortical neurons (Berry & Rogers 1965). However, the ontogenetically oldest neurons of the neo-cortex are located in both the most superficial and deep parts of the cortex, indicating that the neurons of the cortical plate are deposited *in* the marginal zone (Rickmann *et al.* 1977). The oldest neurons are originally oriented horizontally and correspond to *e.g.* Cajal-Retzius cells of Lamina I (Rickmann *et al.* 1977). They are seen in the marginal zone of the occipital region from embryonic day 14 (Rickmann *et al.* 1977).

During development of the neo-cortex there is a rapid formation of synapses and dendrites and proliferation of glial cells resulting in an increased volume of the neuropil. These developmental changes take place mainly during the first 3 – 4 postnatal weeks in the rat (*cf.* Jacobson 1978). The differentiation of neurons in respect of outgrowth of dendrites and spines, follows to a major extent the spatial gradient of deposition of neurons in the cortex. Differentiation starts in the superficial Lamina I, followed by the deep Lamina VI and then successively Lamina V, IV etc. (Schadé *et al.* 1962, Marin-Padilla 1970). During this period different dendrites and portions of a dendrite of a single neuron may show different degrees of differentiation following their laminar distribution (Morest 1969).

A laminar pattern resembling that of the normal cortex develops in tissue culture explants of the neo-cortex of the newborn mouse (Seil *et al.* 1974), suggesting that the differentiation of neurons in the cortex is at least partially independent of the afferent fibres. The synaptogenesis starts before neurogenesis is completed meaning that late-coming neurons will migrate close to and possibly be influenced by neurons which have formed, or are in the process of forming, synapses (*cf.* Jacobson 1978). However, the abnormal migration of neurons in the reeler mouse (Caviness & Sidman 1972, 1973) does not result in abnormalities of the afferent connectivity (Devor *et al.* 1975) or electrophysiology (Bliss & Chung 1979).

It has recently been suggested that 1. inhibition or decreased activation of a neuron may lead to an increase in the number of its dendritic spines and/or postsynaptic sites and 2. activation or decreased inhibition of a neuron may lead to an increase of the number of its axonterminals and/or presynaptic sites (*cf.* Wolff 1979 a, b). GABA, a putative inhibitory transmittor, may induce the formation of dendritic spines and postsynaptic sites in the adult rat (*cf.* Wolff 1979 a, b). The appearance of GABA-accumulating cells in the different Laminae of the visual cortex of the rat follows the same spatial gradient as, but precedes, the differentiation of the neurons in the respective Laminae (*cf.* Wolff 1979 a, b). These findings, which suggest GABA as a morphogenetic factor may to some extent explain both the spatial gradient of the differentiation of neurons in the neocortex and its relative independence of the afferent fibres (*cf.* Wolff 1979 a, b).

A reduction in sensory stimulation during development will reduce the thickness of Laminae II and IV, the number of dendritic spines in Lamina IV and the total number of synapses in *e.g.* the visual cortex illustrating a partial dependence of normal differentiation on the afferent fibres (Gyllensten 1959a, Valverde 1967, 1968, Globus & Scheibel 1967, Cragg 1975). The differentiation of neurons is also influenced by hormones and the nutrition of the animal (see below).

Formation and differentiation of neurons in the cerebellar cortex

The cerebellum is formed by a midline fusion of two parts (*cf.* Altman & Bayer 1978). Fissures are seen from the 20th day of gestation in the rat starting with the primary and posterolateral fissures (Larsell 1952). The mechanism underlying the formation of fissures is not known but before they form there is a local thickening of the external granular layer (Larsell 1952). The external granular cells at the depths of fissures have a higher mitotic rate than those in the crests of lobules (Mares & Lodin 1970). Growth and differentiation of the neurons of the cerebellar cortex show clear tempo-ro-spatial gradients and occur earlier in the archi- and paleo-cerebellum (lobules I – VIa , VIII – X) than in the neo-cerebellum (lobules VIb – VII) (Kuckuk 1967, *cf.* Altman 1972).

Purkinje cells, pale cells and Golgi cells are formed prenatally in the ventricular zone in the rat (*cf.* Altman & Bayer 1978). The Purkinje cells form a mono-layer below the external granular layer at, or shortly after, birth (Kuckuk 1967). The outgrowth of Purkinje cell dendrites takes place mainly during the second and third postnatal weeks and is preceded by the formation of the apical cones of the Purkinje cells (*cf.* Altman 1972). The external granular layer consists of cells which appear to originate from the "germinal trigone" and spread over the surface of the cerebellum (*cf.* Altman & Bayer 1978). Most interneurons – granule, basket and stellate cells – are formed in the external granular layer during the first three or four postnatal weeks (*cf.* Altman 1972). The granule cell migrates to the inner granular layer leaving its axon *i.e.* the parallel fibre in the molecular layer (*cf.* Altman 1972). Basket and stellate neurons do not migrate but remain in the molecular layer (*cf.* Altman 1972).

A few synapses are seen in the thin molecular layer of the cerebellar cortex of the rat already before birth (West & del Cerro 1976). Climbing fibres form transitional synapses on somatic spines of the Purkinje cells during the first postnatal week (*cf.* Altman 1972). From the third week, the Purkinje cell soma makes synaptic contacts mainly with basket cell axons. The differentiation of the neurons in the molecular layer and the formation of synapses on the Purkinje cell dendrites occur in an inside-out fashion (*cf.* Altman 1972). The differentiation of the neurons in the granular layer shows no differences between deep and superficial parts (*cf.* Altman 1972).
The hypothesis that the formation of postsynaptic sites and the growth of dendrites may be induced by increased inhibition or decreased activation gains further support by recent studies on the cortex of the cerebellum (*cf.* Wolff 1979a, b). The Purkinje cell dendritic spines are formed during development also when climbing fibres are lacking and a destruction of the climbing fibres in the adult rat leads to a de novo formation of spines (*cf.* Sotelo 1978). These effects may well be mediated by GABA which is accumulated and synthesized by neurons in the cortex of the cerebellum (Hökfelt & Ljungdahl 1970, 1972, Chan-Palay *et al.*1979).

Development of oxidative metabolism

During development there is rapid increase in the activities of various enzymes involved in the oxidative degradation of glucose for the production of high energy compounds in the brain (*e.g.* succinic acid dehydrogenase, SDH; cytochrome oxidase, CYO) (*cf.* Seiler 1969). Lactic acid dehydrogenase (LDH)-isoenzymes show a shift from anaerobic to aerobic forms (Bonavita *et al.* 1964). These alterations appear to be due to an increase in the enzymatic activity, and possibly also in the number, of mitochondria (Milstein & Swaiman 1967, Dahl & Samson 1959, Samson *et al.* 1960). Many findings suggest that some developmental changes in the oxidative metabolism are correlated to the formation of synapses. There is a high number of mitochondria in mature synapses (Palade 1958) and mitochondria in the synaptosomal fraction have high SDH-activity (Balazs *et al.* 1966). The changes in oxidative metabolism during development follow the same temporospatial gradients as the differentiation of neurons and formation of dendrites and synapses in the cortex of the cerebrum and cerebellum (*cf.* Friede 1966, Seiler 1969, Farkas-Bargeton & Diebler 1978). The formation of dendrites and dendritic spines appears to precede the appearance of SDH-activity in the neuropil (Farkas-Bargeton & Diebler 1978).

Some aspects on the development of neuroglia

The development and adult function of glial cells are closely related to those of neurons (*cf.* Jacobson 1978). Proliferation of glial cells in tissue culture is dependent on and proportional to the addition of neurons (Mc Carthy & Partlow 1976). The number of glial cells increases in both the cerebral and cerebellar cortex during the period of differentiation of neurons (*cf.* Gyllensten 1959a, Das 1976). Glial proliferation is seen *in vivo* concomitant with chromatolysis of neurons following the cutting of their axons (Sjöstrand 1965, 1966). Astrocytic processes also respond to an increased activity in neurons (Güldner & Wolff 1976). The role of the astrocyte for the nutrition of neurons has long been speculated upon (*cf.* Wolff 1970). Recent studies indicate glial cells as a source of nerve growth factor (Burnham *et al.* 1972, Varon *et al.* 1974). Glioblasts and astrocytes take up and release GABA (Hökfelt & Ljungdahl 1970, 1972, Minchin & Iversen 1974, Sellström & Hamberger 1977, Wolff *et al.* 1979). Glial cells guide the migration of neurons in both the cerebral and the cerebellar cortex (Rakic 1971, 1972, Das *et al.* 1974, Smechel & Rakic 1978).

Hormonal influences on the morphogenesis of the brain

Thyroid hormone appears to induce or accelerate the differentiation of neurons during development via nuclear receptors (*cf.* Legrand 1977, Jacobson 1978). Hyperthyroidism from birth results in an accelerated differentiation of neurons in the rat while thyroid deficiency results in *e.g.* a delayed outgrowth of the dendrites of the Purkinje cells and prolonged proliferation in and persistence of the external granular layer (*cf.* Legrand 1977).

An increased DNA-content of the brain has been described in perinatal rats born of mothers which were injected with growth hormone (Zamenhof *et al.* 1974). Growth hormone increases the size and the DNA-content of the placenta but does not pass the placental barrier (*cf.* Legrand 1977). The increase in DNA of the brains of the offspring may be ascribed to improved nutrition of the foetus or associated hormonal alterations (*cf.* Legrand 1977). Injected growth hormone prolongs gestation and foetuses delivered near term by caesarian section show no increase in the DNA of the brain compared with age-matched controls (Croskerry *et al.* 1973). Early hypophysectomy in rats appears not to affect the development of the brain (*cf.* Legrand 1977).

Sex hormones appear to influence mainly the development of the sexual dimorphism of the brain (*cf.* Jacobson 1978). The production of glucocorticoids is low during postnatal development in the rat and these hormones appear to be of low physiologic significance for the morphogenesis of the brain (*cf.* Legrand 1977). Injected adrenal glucocorticoids may affect both the formation of glial cells and the differentiation of the neurons (*cf.* Legrand 1977).

DEVELOPMENT OF THE BRAIN IN PROTEIN-CALORIE MALNUTRITION (PCM)

Formation and migration of neurons in the cerebral cortex

A reduced content of DNA in the brain has been demonstrated in rats on embryonic day 20 and at birth using experimental methods of PCM comparable to that of the present study (Zamenhof *et al.* 1968, 1971, Zeman & Stanbrough 1969, Winick 1969, 1970a, b, Koshy *et al.* 1975). No decrease was found in the DNA content of the forebrain of new-born rats after a 50 per cent reduc-

tion of maternal food intake from day 5 of the gestation (Patel *et al.* 1973); however, a reduced thymidine labelling index of the ventricular cells suggests a concomitant reduction of the mitotic activity and the physiological cell death (Lewis *et al.* 1975). Shrader and Zeman (1969) described a slight reduction of small neurons in Lamina II and III and of larger multipolar neurons in the deeper Laminae of the cortex of new-born rats after PCM. These studies indicate a reduced formation of cells in the brain after PCM during the prenatal period. Reduced physiological cell death may to some extent compensate a reduced formation of cells. The decreased DNA-content of new-born rats after PCM has been thought to affect mostly the neurons of the cortex. The basis for this assumption has been the view that the majority of cells in the cortex at this age are neurons (Brizzee *et al.* 1963). The studies have, however, been performed on whole hemispheres thus including the white matter and the subcortical gray matter (*cf.* Sourander *et al.* 1974). The most marked reduction in thymidine labelling index after maternal PCM is according to Winick (1970a) seen in the ventricular zone hence the reduction may affect also the formation of glioblasts. The results of Shrader and Zeman (1969) and of studies of others on prenatally formed neurons of the cerebellar cortex (see below) support the view that the number of neurons may be affected by maternal protein deprivation during gestation.

Studies on the migration of cerebral cortical neurons in PCM have not been reported.

Differentiation of neurons in the cerebral cortex

The differentiation of neurons in PCM has been described with regard to (1) structural aspects *e.g.* the growth of dendrites and the formation of synapses, (2) chemical aspects *e.g.* protein synthesis and metabolism of transmittor substances and (3) functional aspects *e.g.* evoked potentials. Several problems adhere to behaviouristic studies since the test situation may differently influence control and PCM animals and since an altered behaviour of PCM animals may also be ascribed to alterations in their social environment (*cf.* Morgane *et al.* 1978, Crnic 1970, Massaro *et al.* 1974).

Structural aspects

A decrease in the thickness of the cerebral cortex has been described in young and adult rats after different types of experimental PCM (*cf.* Morgane *et al.* 1978). Results on the size of the neurons in the cerebral cortex differ with descriptions of both decreased (Callison & Spencer 1968, Stewart *et al.* 1974, Angulo-Colmenares *et al.* 1979) and un-altered cell size (Gambetti *et al.* 1974). Oldfors and Sourander (1977) found no differences in size of spinal ganglion cells of normal and PCM rats.

Increased cellular density of the cerebral cortex in PCM was first described by Sugita in 1918 and this finding has been corroborated by various authors (Eayrs & Horn 1955, Callison & Spencer 1968, Siassi & Siassi 1973, Gambetti *et al.* 1974, Cordero *et al.* 1976). The increased cellular density is due probably to the lesser volume of the neuropil per cent of the cortical volume (Gambetti *et al.* 1974, Colmenares 1976, Angulo-Colmenares *et al.* 1979) resulting from decreased formation of nerve cell processes and reduced size and number of glial cells (see below). A reduction in the length and thickness of nerve cell dendrites and in the number of synapses per volume has been described in the cerebral cortex after PCM (Cragg 1972, Gambetti *et al.* 1974, Salas *et al.* 1974, Stewart *et al.* 1974, Cordero *et al.* 1976, West & Kemper 1976, Dyson & Jones 1976a, b,

Angulo-Colmenares *et al.* 1979). An ultrastructural study of synapses has demonstrated an immaturity of synapses in the adult cerebral cortex after early induced chronic PCM (Dyson & Jones 1976a, b).

When these alterations first become manifest can not be decided due to lack of studies on perinatal development. Altered differentiation of dendrites in PCM was seen in rats 7 days old by Salas *et al.* (1974; younger rats were not examined). The studies by Dyson & Jones (1976a, b) also indicate a reduced density of synapses in the molecular layer of the visual cortex of the rat on the 7th postnatal day (younger rats were not examined).

Sima (1974) described a reduction in the diameter of axons in the peripheral nerves and nerve roots of young and adult rats after PCM. This and other studies indicate greater influences of PCM on the sensory than on the motor fibres (*cf.* Sourander *et al.* 1974).

Chemical aspects

Transmittors and transmittor related enzymes: The problem of interpretating studies on transmittor substances and enzymes is that the amount or activity must be either related to the whole brain or expressed *e.g.* per g of the brain or mg of protein. The review includes studies which have used amount or activity per g brain or mg protein etc. since the results of these studies can be more easily compared to the variables of the present study which are expressed per volume of tissue.

As mentioned previously GABA is discussed not only as a putative inhibitory transmittor substance but also as a morphogenetic factor (*cf.* Wolff 1979a, b). Banay-Schwartz *et al.* (1978) found normal amounts of GABA in rats aged 0, 6 and 12 days but reduced amounts at 30 and 42 days of age after early induced PCM. Dickerson and Pao (1975) found un-altered concentrations of GABA in the forebrain of protein deprived rats at 0 and 21 days postnatally. PCM reduces the normal increase in the quotient between glutamine and glutamic acid indicating an impaired compartmentation of amino acids (Patel & Balazs 1970, Roach *et al.* 1974, Patel *et al.* 1975). Altered activity of enzymes related to the metabolism of GABA has been described in PCM rats (Rajalakshmi *et al.* 1969, 1974, Katiyar *et al.* 1976, Chase *et al.* 1976). The activity of glutamic acid dehydrogenase and decarboxylase was reduced at 21 days postnatally in rats subjected to PCM by an increased litter size (16 – 8) mostly due to a reduction of the normal developmental increase during the second postnatal week (Rajalakshmi *et al.* 1974). The enzymatic changes described by Katiyar *et al.* (1976) would theoretically lead to an increased synthesis of glutamic acid from glutamine and from glutamic acid to GABA leading to increased amounts of GABA in the cortex. A decreased or unaltered amount of GABA in PCM brain may possibly be due to increased degradation of GABA by enzymes related to the Kreb's cycle (McKhann *et al.* 1960, *cf.* Swaiman 1970).

Studies on other putative transmittors indicate increased concentrations of serotonin and of noradrenaline in protein deprived rats at birth and 21 days postnatally. Increased concentrations of serotonin and 5-hydroxy-indole acetic acid in the brain after protein deprivation are probably due to the increased concentration of the precursor tryptophane in the brain and the blood plasma (Morgane *et al.* 1978).

Acetylcholine has not been investigated due to its rapid postmortem degradation. Acetylcholinesterase is not or only slightly affected in PCM rats up to 3 weeks postnatally (Adlard & Dobbing 1970, 1972, Im *et al.* 1971, Gambetti *et al.* 1972, Sobotka *et al.* 1974). Protein deprived rats of 7 weeks and adult rats rehabilitated nutritionally after early PCM show an increased activity of acetylcholinesterase in the brain (Adlard & Dobbing 1972b, Im *et al.* 1971). The activities of cholinesterase and acetylcholinesterase in nerve endings isolated from the brains of adult protein deprived rats is increased or unaltered (Morgane *et al.* 1978). This finding may be influenced by an altered distribution of nerve endings in the fractions (Gambetti *et al.* 1972).

Kissane and Hawrylewicz (1975) found decreased amounts of Na^+/K^+-ATPase per mg protein in synaptosomal fractions of brains of 14 and 21 day old PCM rats.

Protein metabolism: Several methods have been described for the determination of the rate of protein synthesis by measurements of the incorporation of radioactive labelled amino acids into proteins (*cf.* Nowak & Munro 1977). The various techniques are influenced by factors such as alterations in the uptake and concentration in the free amino acid pool of other amino acids due to increased concentration of the labelled amino acid administered (*cf.* Nowak & Munro 1977).

Patel *et al.* (1975) described decreased incorporation of leucine into protein in the brains of 15 and 20 days old PCM rats. Patel *et al.* (1975) in contrast to Dainat (1974) and Morgane *et al.* (1978) made corrections for the concentration of unmetabolized leucine which may be suspected to be increased in PCM rats due to decreased degradation via the tricarboxylic cycle (Roach *et al.* 1974). Injected leucine affects, however, the concentration of other amino acids in the brain (*cf.* Nowak & Munro 1977).

The amino acid valine was used by Hamberger and Sourander (1978) for the determination of proteinsynthesis in the brain and in nerve cell and glial cell fractions. No effects of valine on the concentration of other amino acids in the brain have been described. The incorporation of valine into protein in the cerebral cortex of PCM rats was decreased at 11 days, increased at 34 days and unaltered at 21 and 90 days postnatally compared with controls. The same differences were seen in the nerve cell fraction (Hamberger & Sourander 1978). The concentration of valine in the cerebral cortex was, however, lower in the PCM rats at all ages examined. Banay-Schwartz *et al.* (1978) found a reduced incorporation of tyrosine in PCM rats at 6 days and unaltered at 12 and 42 days postnatally. Net deposition and break down of protein were decreased in PCM rats at 6 and 12 days indicating decreased synthesis and turnover of proteins. Net deposition of protein was increased in 30 day old PCM rats.

The present results indicate a decrease in the rate of protein synthesis in PCM rats during early postnatal development. At approximately 30 days postnatally there is probably an increased synthesis and net deposition of proteins. The break-down of protein is decreased in the brains of PCM rats during postnatal development.

Haltia (1970) demonstrated a reduced RNA-content of anterior horn cells during postnatal development in severe PCM caused by a reduced suckling time (reduction of body weight 90 per cent). Sourander *et al.* (1976) found unaltered amounts of RNA per anterior horn cell before, and a decrease during a period after, weaning in PCM induced by a 50 % reduction in the daily intake of food. Decreased synthesis of cytoplasmic RNA in the brains of PCM rats was demonstrated by de Guglielmone (1974) who also demonstrated *in vitro* a decreased synthesis of nuclear RNA in 10 and 20 but not in 30 days old PCM rats.

Significant alterations in the free amino acid pool have been shortly presented in the section on transmittor substances.

Energy metabolism: Postnatal PCM does not significantly alter the oxygen consumption by mitochondria *in vitro* (Muzzo *et al.* 1973). Mitochondria from the brains of pre- and postnatally protein deprived rats show a reduced capacity for the synthesis of ATP but a concomitant increase of ATP in relation to ADP (Morgane *et al.* 1978). The concentration of creatine phosphate, glucose and glycogen but not of ATP is increased in the brain by early PCM (Culley 1971). Sereni *et al.* (1966) found no alterations in the activity of succinic acid dehydrogenase per nitrogen after postnatal PCM. The activity of the LDH-isoenzymes expressed per mg protein is un-altered during postnatal development by PCM (Swaiman & Wolfe 1970, Morgane *et al.* 1978). These findings indicate a normal energy metabolism in relation to growth in the brain of PCM rats (*cf.* Morgane *et al.* 1978).

Functional aspects

Reported studies on evoked potentials in PCM rats have shown increased latencies in the visual, auditory and somatosensory cortex before and shortly after weaning (Mourek *et al.* 1967, Callison & Spencer 1968, Zahlava & Myslivecek 1968, Salas & Cintra 1973). Morgane *et al.* (1978) found increased latencies of the visual evoked potentials of protein deprived rats at 14 and 20 but not at 90 days postnatally. Responses after stimulation of the contralateral cortex also showed increased latencies. The differences, thus, were probably not due to alterations in the sensory organ (Morgane *et al.* 1978).

Formation and migration of neurons in the cerebellar cortex

Morphological studies on the formation, migration and differentiation of neurons of the cerebellar cortex are facilitated by the localization of the different types of neurons in distinct layers.

Several morphometrical studies on cell number in the cerebellar cortex have been reported. Due to earlier discussed difficulties associated with quantitative studies on DNA-content as a tool for the study of cell number only the results of morphometrical studies will be presented. The results of the biochemical and morphometrical studies are in agreement (Chase *et al.* 1969, Fish & Winick 1969a, b, Gourdon *et al.* 1973).

Reduced numbers of Purkinje, granule, basket and stellate cells have been described after early induced PCM (Dobbing *et al.* 1971, Barnes & Altman 1973a, b). The time of the mitotic cycle of the cells in the external granular layer is increased in PCM rats (Deo 1978). Studies on cell death in the external granular layer have given divergent findings. Lewis (1975) found increased cell death in PCM rats but this could not be demonstrated by Deo (1978). Granule, basket and stellate neurons *i.e.* the neurons that are formed mostly postnatally, show greater reduction in number than do the prenatally formed Purkinje cells after pre- and postnatal PCM (*cf.* Shoemaker & Bloom 1977). This fact may be explained either by (a) differences in sensitivity of the respective precursor cells, (b) increased effectiveness of the PCM during the suckling period or (c) decreased proliferation of the cells in the external granular layer *e.g.* due to an impaired differentiation of Purkinje cells (see below).

No studies concerned with the migration of Purkinje cells have been reported. Deo (1978) found indications of an increased migration time of the granule cells in PCM rats. The results were, however, influenced by the decreased formation of the granule cells. The migration of the granule cells is influenced both by Purkinje and Golgi epithelial cells (Rakic 1971, Das *et al.* 1974).

Differentiation of neurons in the cerebellar cortex

Several studies have reported increased density of neurons and decreased thickness of the various layers in the cerebellar cortex (Chase *et al.* 1969, Dobbing & Sands 1971, Neville & Chase 1971, Dobbing *et al.* 1971, West & Kemper 1976, Clos *et al.* 1977). A delayed morphological and enzymehistochemical development of the cerebellar cortex with special reference to the outgrowth of Purkinje cell dendrites has been reported after early PCM in rats (Sima & Persson 1975, Persson & Sima 1975). Golgi-stained preparations have shown an increased number of abnormalities associated with Purkinje cell dendrites in PCM rats (Griffin & Woodward, *cit.* Shoemaker & Bloom 1977). The dendritic field of Purkinje cells is reduced but the spine density appears to be unaltered (Pysh & Perkins 1975, McConnell & Berry 1978, Stewart *et al.* 1974, West & Kemper 1976). Shoemaker and Bloom (1977) found a 9 % decrease in the number of synapses per unit area in the molecular layer of the cerebellar cortex of PCM rats. Recently, Chen and Hillman (1980) have demonstrated the presence of giant spines of Purkinje cells in 60 day old protein deprived rats. The giant spines were regarded as being similar to hypertrophic spines in the agranular cerebellum of the Weever mutant mouse (Chen & Hillman 1980).

Glial cells

The decrease in cerebral DNA content after PCM during the suckling period is most probably due to a reduction in the number of glial cells (Winick & Noble 1966, Fish & Winick 1969a, b). The reduced DNA content of the cerebellum may be ascribed partially to a reduced number of glial cells (Deo 1978). Also, morphological studies indicating a reduced number of astroglial cells and glial cell processes have been reported (Siassi & Siassi 1973, Clos *et al.* 1973). Protein synthesis in the glial cell fraction in the study reported by Hamberger & Sourander (1978) was decreased at 11 days and unaltered at 16, 21, 34 and 90 days postnatally in PCM rats. Reduced amounts of the S–100 protein have been described in certain brain regions after PCM (Moore *et al.* 1977). Alterations related to oligodendrocytes and myelination have been reported in several biochemical as well as morphological studies (Dobbing 1964, Benton *et al.* 1966, Bass *et al.* 1970, Sima & Sourander 1973, Sourander *et al.* 1974, Robain & Ponsot 1978).

Blood vessels

Siassi and Siassi (1973) have reported a reduced endo-glial index (the number of nuclei of endothelial cells and glial cells over the number of nuclei of neurons) in the cerebral cortex of PCM rats.

AIMS OF THE INVESTIGATION

The present study was undertaken to examine

1. the formation and branching of stem vessels in the embryonic brain of normal and protein deprived rats (paper I),
2. the formation and branching of stem vessels in the cerebellum of normal rats (paper II),
3. the growth of vessels in the cerebral and cerebellar cortex of normal and protein deprived rats (papers II, III, IV).

MATERIAL AND METHODS

Animals

The present study was carried out on the offspring of Sprague-Dawley rats which were always obtained from the same breeder (Anti-Cimex). The rats were mated over night. The day of the first sperm positive vaginal smear was taken as embryonic day 1 (E 1). Delivery usually occurred on day E 23. At birth each litter was limited to 7 or 8 pups retaining as many male pups as possible. The animals were kept in plastic cages at an ambient temperature of + 22°C and an air humidity of 60 per cent. The animal room was illuminated 12 hours daily in a normal day and night sequence. The cages were cleaned once weekly. Both sexes were used for studies on prenatal development. Only male rats were used for studies on postnatal development since sex dependent differences in development cannot be excluded.

Diets

Two diets were used, the composition of which is shown in Table I. The protein content of the respective diets has been expressed in weight per cent corresponding approximately to 16 (Normal protein, NP) and 8 (Low protein, LP) calorie per cent. Calorie per cent, being the more common way of expressing the amounts of protein in diets is based on assumptions concerning the content of calories of the dietary constituents. The diets were made iso-caloric by supplementing the low protein diet with starch. Both diets contain adequate amounts of minerals and vitamins (Mc Coy 1963). The diets were prepared in our laboratory and administered as pellets. The respective diets were given to female rats from two weeks before mating and during gestation and lactation. The offspring were weaned on the same diet.

Table I. Composition of the diets.

	NP	LP
Weight per cent protein[1]	14	7
Constituents in 25 kg of diet, g		
Protein (SP-protein, Swedish Protein AB)	4.460	2.230
Wheat starch	15.500	17.700
Sucrose	1.300	1.300
Hydrogenated lard	1.950	1.950
Sunflower oil	415	415
Linseed oil	150	150
Cellulose	900	900
Salt mixture[2]	1.000	1.000
Choline chloride	50	50
Vitamine mixture[3]	37	37

1. Calculated on the basis of crude protein content: 80 per cent fish protein according to manufacturer (Swedish Protein AB).
2. Salts in 25 kg of diet: $MnSO_4 \cdot 7H_2O$ 4.0 g; NaCl 139.3 g; KH_2PO_4 389.0 g; $MgSO_4$ 57.3 g; $CaCO_3$ 381.4 g; $FeSO_4 \cdot H_2O$ 27.0 g; KI 790 mg; $ZnSO_4 \cdot 7H_2O$ 548 mg; $CuSO_4 \cdot 5H_2O$ 477 mg; $CoCl_2 \cdot 6H_2O$ 23 mg; $KAl(SO_4)_2 \cdot 12H_2O$ 87.95 mg; NaF 276.3 mg; $NaAsO_2$ 8.67 mg; $Na_2B_4O_7 \cdot 10H_2O$ 22.03 mg; $Na_2MoO_4 \cdot 2H_2O$ 3.13 mg.
3. Vitamines in 25 kg of diet: Retinol 300.000 I.U.; Thiamin 1250 mg; Riboflavin 500 mg; Panthotenic acid 2500 mg; Nicotinamide 5000 mg; P-aminobenzoic acid 2500 mg; Naphtoquinone 250 mg; Biotin 25 mg; Folic acid 125 mg; Cyanocobalamin 1.25 mg; Myoinosit 25 g; DL-o-tocopheral 12500 mg; Calciferol 105.000 I.U.; Pyridoxine 500 mg.

Considerations concerning animals and diets

The rat was chosen for the experiments because of its common use in developmental and pathological studies. Its size is convenient for laboratory work. Reproduction is rapid and easily controlled. Direct comparison with human PCM cannot of course be made from this experimental model. Such difficulties apply to all experimental studies in PCM. Nevertheless the findings in PCM rats may suggest pathogenetic mechanisms and paths of investigation for research on human PCM. Several methods of experimental PCM have been described (see Introduction). The method of protein deprivation used in the present study offers certain advantages compared with other methods. The deficiency is controlled and reproducible. Eating and calorie intake are regulated by the animals themselves. Either of two *iso-caloric* diets were given ad libitum and were thus constantly available. Various variables such as handling of the animals are not altered compared to normal breeding of

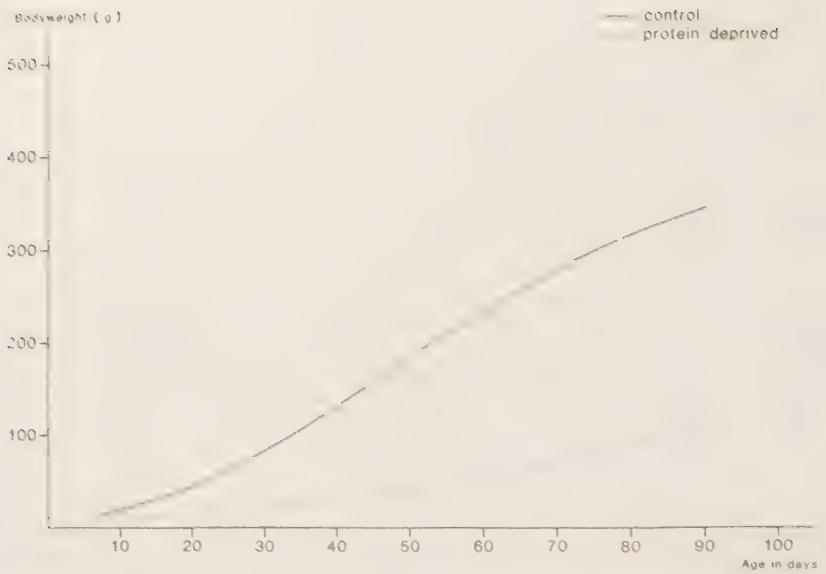


Fig. 1. Body weight of rats (g) during postnatal development (days).

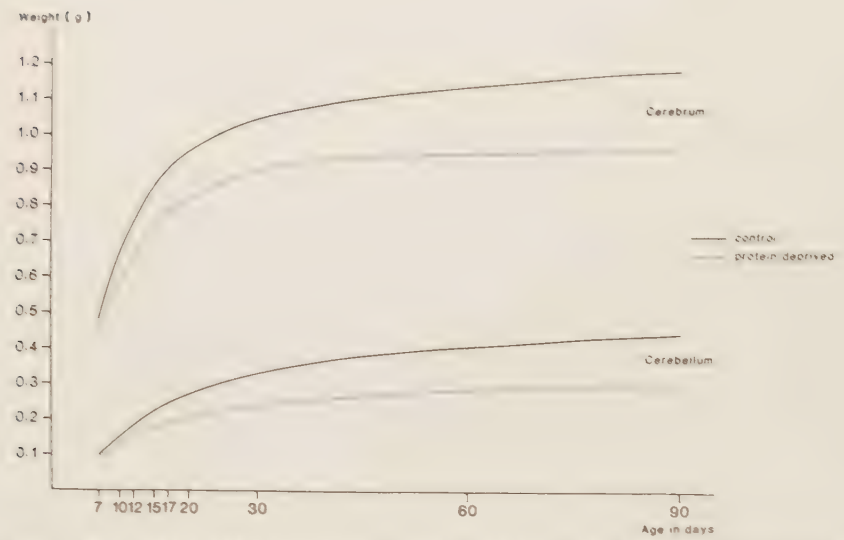


Fig. 2. Weight of the cerebrum and cerebellum (g) during postnatal development (days).

rats. The method may be assumed to give less inter-individual variation than e.g. methods using altered litter size. Compared with methods using a restricted daily intake of pellets the present method is less laborious since food can be administered in amounts sufficient for several days. In the NP and LP diets used by Morgane *et al.* (review 1978) the fat content was doubled compared with that of a normal stock diet. The reason for this is unclear. Other sources of protein than fish have been used (e.g. Morgane *et al.* 1978, casein). The fish protein used in the present study is nutritionally sufficient for rats and no essential amino acids had to be supplemented.

Protein deprivation appeared to influence conception but no difference in number of foetuses was seen during the interval E 12 — 15 between normal and protein deprived mother rats. Protein de-

privation also appeared to reduce postnatal survival rates although this was not statistically evaluated. The protein deprivation resulted in marked reduction in the mean weight of the body, as well as a reduction in the weight of the cerebrum and the cerebellum compared to normal rats (Fig. 1, 2). Protein deprived rats from our laboratory, 6 and 15 weeks old, show moderate reduction in serum contents of albumin and protein compared to normal rats (Oldfors & Ullman 1980).

Morphological studies on vascular development in the embryonic brain (paper I)

Embryonic brains were examined on days E 12, E 13, E 14 and E 15. On each day, 7 to 8 embryos of two normal and of two protein deprived mother rats were included.

Pregnant rats were anaesthetized with 50 mg/kg pentobarbital (Aco). The abdomen was opened, and the uterine vessels ligated. The uterus and the embryos within it were removed and fixed at +37° C over night in 3 % glutaraldehyde and 3 % paraformaldehyde in 0.1 M phosphate-buffer. The following day the embryos were removed from the uterus and from each mother rat, these 3 – 4 embryos showing the best blood-filled vessels were chosen for incubation in Di-aminobenzidine (DAB) and hydrogenperoxide at pH 5.1 according to the method described by Malmgren & Olsson (1976). By this means red blood cells are stained brownish-black due to the oxidative polymerization of DAB by haemoglobin in the presence of hydrogen peroxide. The incubation was performed on whole embryos on day E 12 and 13 and on sectioned heads on E 14 and E 15. The heads were embedded in 5 % gelatine in water. Serial frozen horizontal sections, 100 μm thick, were cut, mounted in glycerine over ice and examined by light microscopy.

The heads of additional embryos, 3 – 4 per mother rat, were dehydrated in a graded ethanol series and embedded in JB4 embedding kit (Sorvall). Sections 2 μm thick were cut at intervals of 100 μm and stained with Azur II and Methylene blue (Richardsson *et al.* 1960).

Considerations concerning the technique

The number of vascular segments containing blood cells was decreased in embryos when the uterine vessels had not been ligated prior to removal. This was probably due to an increased space for blood in the embryonic placental side after emptying the maternal side. Warm fixative was used since this resulted in more blood-filled segments of embryonic vessels. Red blood cells within vessels differ in colour from the nervous tissue. Staining with DAB increased the visibility of red blood cells and facilitated examination. Gelatine embedding did not significantly affect the staining of red blood cells by DAB incubation. Unless embedded in gelatine the thin walls of the embryonic brain fragmented easily with loss of morphological detail. Neither embedding nor freezing of the material during sectioning altered the staining capacity of the red blood cells. Although some segments of vessels were empty the angioarchitecture could be described adequately from the blood filled segments in combination with the results obtained from plastic sections. The use of plastic sections permitted localization of the segments of vessels to the various zones of the wall of the embryonic brain. Comparison of the size of the brain, development and relative thickness of the wall zones was carried out on both types sections.

Morphological studies on vascular development in the cerebellum (paper II)

Rats were examined during prenatal development on days E 18, E 19, E 21 and E 23 and during postnatal development at the ages 1, 5, 10, 15 and 90 days.

Pregnant rats were anaesthetized with 50 mg/kg pentobarbital (Aco). The abdomen was opened and the foetuses removed one by one. The abdomen was irrigated with warm Ringer solution during the experiments. Rats during postnatal development were anaesthetized with ether. The technique for demonstration of blood vessels was the same for pre- and postnatal material. The thoracic cage was opened and the heart exposed. Animals were killed by opening of the right atrium and perfused through the heart with the following solutions: 1. Rinsing media: Perfadex (Pharmacia) at room temp. perfused for approximately 30 sec. 2. Fixative: 4 % paraformaldehyde in 0.1 M phosphate buffer at room temp. perfused for approximately 5 min. 3. Ink-mixture: Colloidal carbon (C 11a/1431, Günther Wagner Werke) mixed with an equal volume of 5 % gelatine in water at +37°C perfused for approximately 5 min. After the perfusion the animals were stored at −15°C for 5 min. to obtain a rapid hardening of the gelatine. Heads of foetuses and brains of rats during postnatal development were postfixed in the fixative. A central specimen of the foetal cerebellum or of the vermis 3 – 4 mm thick was cut with a razor blade. Specimens of the foetal cerebellum were embedded in gelatine prior to sectioning. Serial frozen sections 100 μm thick were cut from side to side and mounted in glycerine. Additional sections 20 μm thick were stained with toluidine for morphological examination of the cerebellar cortical layers.

Considerations concerning the technique

The short perfusion fixation was used to stabilize the vessel walls and surrounding nervous tissue. This has been found to improve the filling of vessels especially in foetal rats (Wolff personal communication). Pfeiffer (1928) was extremely cautious about the completeness of the ink injection. However, sections with incomplete filling of vessels were found to give valuable information *e.g.* on the branching patterns of pial and stem vessels which were more easily described after incomplete filling since the dense capillary network often tended to obscure these details. The angioarchitectonics could be more easily defined in the 100 μm thick sections than in sections 20 μm or 200 – 300 μm thick. Embedding in gelatine was used to decrease the tendency to fold of sections through the cerebellum of foetal rats.

Morphometrical studies on the vascular development in cerebral and cerebellar cortex (papers III, IV)

A total of 62 rats were used for the morphometrical studies on blood vessels in the cerebral and cerebellar cortex. The ages 7, 10, 12, 15, 17, 20, 30 and 90 days were examined. Rats were anaesthetized with ether, the thoracic cage opened and animals killed by bleeding by a cut into the right atrium. The vascular system was rinsed briefly by perfusion with 0.1 M phosphate buffer containing 5 % sucrose . Animals were fixed by perfusion with 3 % glutaraldehyde and 3 % paraformaldehyde in 0.1 M phosphate buffer. The time of perfusion was 30 min., the volume in cc being the same as (90 day old rats) or twice (7 - 30 day old rats) the body weight in grams. The brains were removed and postfixed in the same fixative for 4 hours. The specimens were rinsed over night, dehydrated in graded ethanol series and embedded in JB4 embedding kit (Sorvall). Sections 2 μm thick were stained with Azur II and Methylen blue according to the method of Richardsson *et al.* (1960). Four consecutive rows of ten consecutive field areas through the neo-cortex of area 18 (Krieg 1946) were examined on 1 section 2 μm thick from each animal. Field areas with radial vessels were avoided since the capillary density is low in the vicinity of such vessels (Saunders *et al.*, 1971).

Three consecutive rows of 3 field areas – the granular layer, the Purkinje cell layer and the molecular layer – through the cortex of lobule IX of the cerebellar vermis were examined on three sections per animal. To avoid systematic error the distance between sections was kept at approximately 25 μm.

Light microscopic images of the sections were scanned by a TV camera. The optimal optical magnification (60 or 80x) for the cerebral cortex was decided automatically from data on cortical thickness. The size of the field area was one tenth of the cortical thickness times its width being constant (175 μm). A constant field area of 55 μm x 137.4 μm was used for the cerebellar cortex. The vascular variables (see below) were measured by an automatic TV-analyzer Quantimet 720 (Metals Research, Cambridge) connected to a Hewlett-Packard 9830 desk top calculator and a Texas Instruments' Silent 7000 printer.

The specific length (Lv), specific surface (Sv) and the volume fraction (Vv) of vessels were determined from the number, total circumference and total area of the vascular profiles per field area using the contrast differences between empty vascular lumina and stained surrounding tissue. To obtain the diameter of the lumen of the vessel the following procedure was employed. The area of a circle over that of a square containing it is $\pi/4$. A rectangular frame was placed so as to contain the lumen of the vessel and rotated in steps of 45°. Where and when the area of the lumen of the vessel over that of the rectangle was approximately $\pi/4$ the shorter side of the rectangle was taken as the diameter of the lumen. Orientation of vessels in the tissue was determined by the axial ratios of the elipses formed by the lumina of blood vessels in the section. Random orientation, being a pre-requisite for the employed morphometrical technique, was checked by comparing the axial ratios of sectioned vessels with those expected for randomly orientated cylinders (Eins & Bär 1978). The section thickness was not included in the calculations due to the favourable relation between the thickness and the vascular variables. Tissue shrinkage was determined by measuring the specimen area during immersion fixation and after dehydration (Eins & Wilhelms 1976). A factor compensating for shrinkage (K) could thus be calculated from the specimen area during fixation divided by the area after dehydration. Compensation for tissue shrinkage could thus be made by Lv/K (dimension $1/mm^2$) Sv/$\sqrt{K}$ (dimension 1/mm) and no compensation for Vv (dimensionless). Statistical evaluation was performed with Fisher's permutation test and differences were regarded as significant when $P < 0.05$.

Considerations concerning the technique

The pressure of the perfusion fixation may influence the diameter of lumina, Sv and Vv of vessels. These variables showed larger interindividual variations than did the Lv of vessels although perfusion pressure was kept constant. Tissue shrinkage due to dehydration may increase, or may leave unaltered or decrease the diameter of the lumina of the vessels. The Sv and Vv may be affected by tissue shrinkage more than what can be compensated for by the correction factor but an attempted evaluation of this possibility did not give a conclusive answer. The Lv is thus probably the variable least influenced by the experimental technique. Data on the diameter of lumina, Sv and Vv should be interpreted with care.

RESULTS

Formation and branching of stem vessels in the embryonic brain

No differences were seen for the timing of events of the internal vascularization or the morphological maturation of the brain between embryos of normal and protein deprived mother rats. There was also no difference between the two groups neither with regard to body weight and size of the brain of embryos nor the number of embryos per mother rat.

A single set of stem vessels were seen in the embryonic brain in regions where the marginal zone was present. The marginal zone extended only for a short distance from vascularized regions. The formation of this first set of stem vessels and of the marginal zone was thus temporally and spatially correlated. The stem vessels of this first set branched and terminated in the deep parts of the ventricular zone. The branches were fairly parallel to the ventricular surface. The distance of the branches from the ventricular surface was approximately twice or three times that of the nuclei of the germinal cells (stage I, Fig. 3). After the formation of the intermediate zone branches of new stem vessels formed a vascular plexus in the superficial parts of the ventricular zone (stage II, Fig. 3). Stem vessels of a third set were seen to terminate in the subventricular and intermediate zones during the subsequent development (stage III, Fig. 3).

The stem vessels were seen in parts of the rhomb-, the mes- and the prosencephalon on E 13. The gradient of the internal vascularization proceeded from the medial to the lateral parts in the rhombencephalon and from the inferior and lateral parts towards the posterior and superior parts in the mesencephalon. The diencephalon became vascularized first in the inferior and posterior parts and subsequently in the more anterior and superior parts. The internal vascularization of the telencephalon proceeded from parts adjacent to the ganglionic eminence in the floor of the lateral ventricle towards anterior superior parts. The first set of stem vessels in the medio dorsal angle of the telencephalon were not seen until E 15. This part of the telencephalon was in its anterior medial extension continuous with the area choroidea where no vessels were seen to enter (Fig. 4).

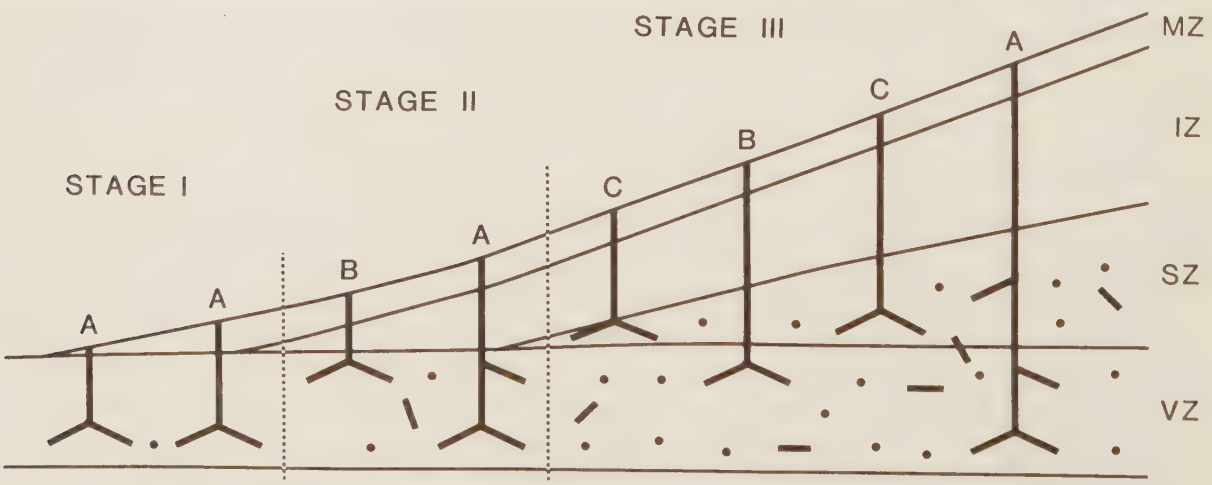


Fig. 3. Schematic illustration of the approximate spatial and temporal relations between formation and termination of stem vessels and the wall zones. The sets of stem vessels A and B are formed early and terminate in deep and superficial parts of the ventricular zone (VZ) respectively. Stem vessels of set C are formed later and terminate outside the ventricular zone. (MZ = Marginal zone, IZ = Intramediate zone, SZ = Subventricular zone).

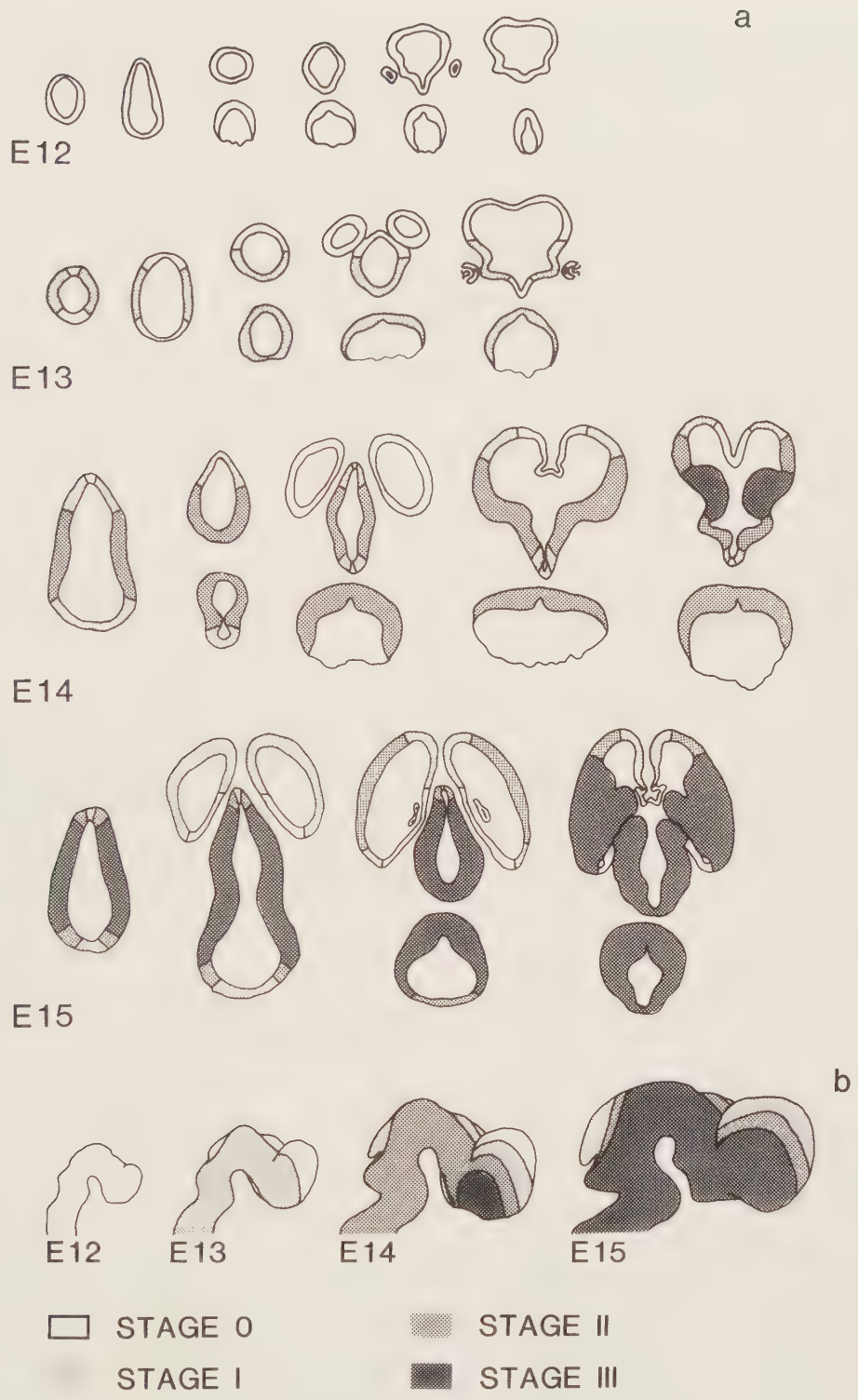


Fig. 4. Schematic illustration of the internal vascularization as seen in a) horizontal sections through and b) lateral view of E 12, 13, 14 and 15 embryonic brains. The interval between sections in a is 100 µm in E 12 and 200 µm in E 13–15. Compare with stages in Fig. 3.

Formation and branching of stem vessels in the cerebellum (paper II)

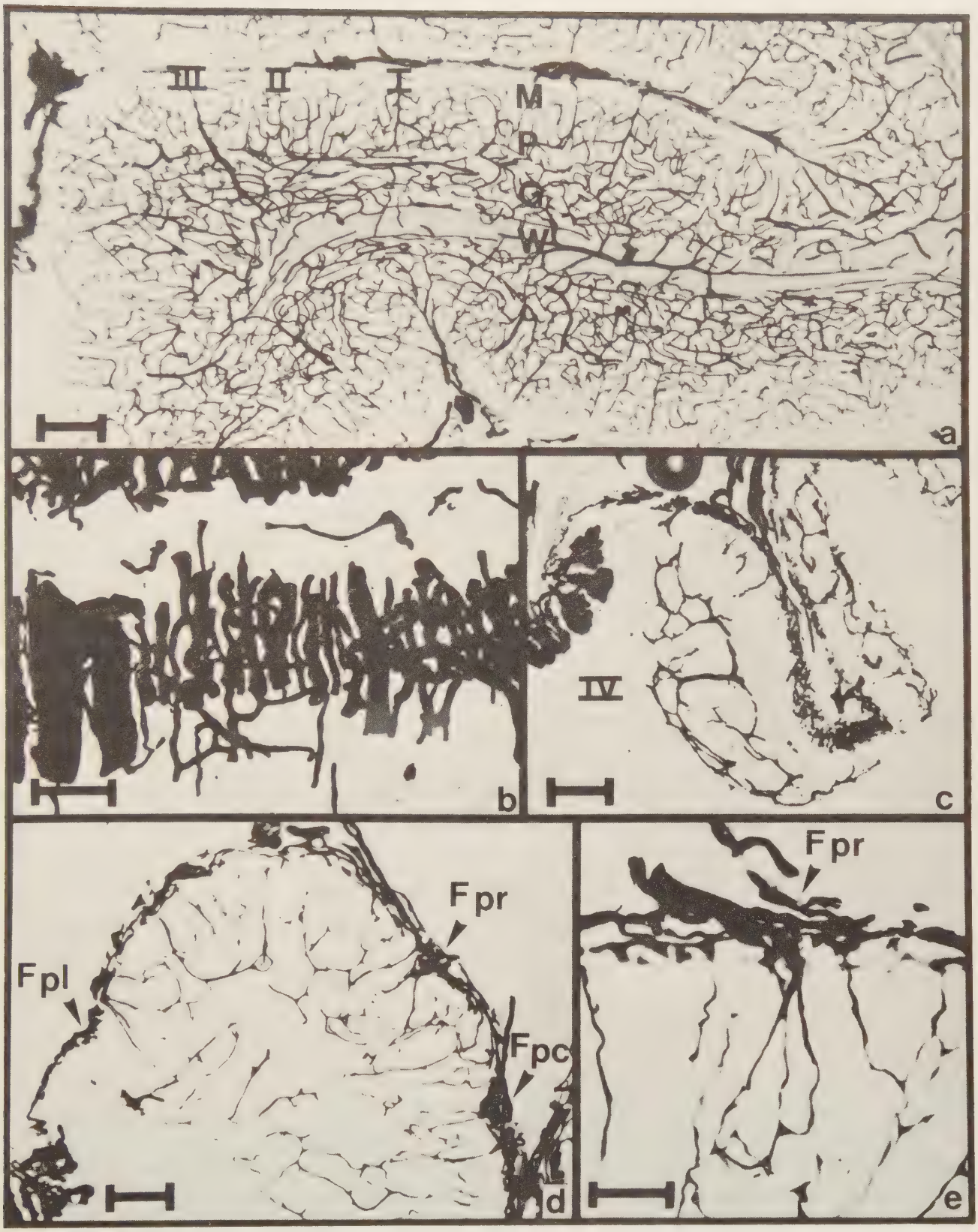
The distribution of stem vessels and branches of vessels in the cerebellum on days E 18 and 19 corresponded to that described in the embryonic brain. Additional stem vessels were formed during subsequent development. Branches of vessels were seen in successively more superficial parts of the cerebellum. Some stem vessels branched in the Purkinje cell layer of 1 day old rats (Fig. 5). Short stem vessels, being approximately of capillary size were seen in the superficial parts of the molecular layer in 15 day old rats.

The distribution and formation of stem vessels showed regular differences during development. Fissures and sulci were seen as successively deeper indentations in the cerebellar surface starting with the primary and the preculminate fissure on E 21 (E 19). The number of stem vessels entering the cerebellum was clearly higher at the shallow indentations of early fissures and at their depths when they got deeper than the number entering at other parts of the surface of the cerebellum. This pattern was seen at all ages including the adult animals (Fig. 5). Stem vessels terminated superficially in the cortex earlier in the paleo-cerebellar than in the neo-cerebellar parts. Terminations of stem vessels were seen up to the middle parts of the molecular layer in paleo-cerebellar parts whereas the molecular layer of neo-cerebellar parts was vascularized only in a zone close to the Purkinje cell layer in 15 day old rats.

Stem vessels in the adult cerebellum branched and terminated mainly at three levels: the white matter, the Purkinje cell-granular layer and the molecular layer. The stem vessels gave off smaller branches on their way through more superficial parts of the cortex. The stem vessels often turned slightly towards the centre of the cerebellum. Larger vessels followed the direction of the fibres in the white matter. Numerous stem vessels entered the cortex at the depths of fissures and sulci.

Vessels in the pial plexus of fissures showed a markedly parallel arrangement from the early postnatal period (Fig. 5). These vessels gave off perpendicular side branches to both sides *i.e.* entering the lobules on either side of the fissure.

Fig. 5. ***a.*** *Parasagittal section through Lobule VIII of adult cerebellar vermis. Note perpendicular stem vessels terminating in different levels (I–III) and radiating pattern in the depth of the fissure (right) and the sulcus (bottom). (M = Molecular layer, P = Purkinje cell layer; G = Granular layer; W = White matter; Bar = 200 µm).* ***b.*** *Coronal section through cerebellum of a 10-day-old rat. Note the parallel course of most vessels in the tangentially cut pial plexus. (Bar = 100 µm).* ***c.*** *Parasagittal section through an E 18 cerebellum. The choroid plexus of the fourth ventricle (IV) is seen at the left (Bar = 200 µm).* ***d.*** *Parasagittal section through an E 21 cerebellum. Note the stem vessels arising in depths of early fissures. (F.pr. = Primary fissure; F.pl. = Posterolateral fissure; F.pc. = Preculminate fissure; Bar = 200 µm).* ***e.*** *Parasagittal section of an E 21 cerebellum. Note numerous stem vessels at the Primary fissure (F.pr.). (Bar = 100 µm).*


III
II
I
M
P
G
W
a
b
IV
c
Fpr
Fpl
Fpc
d
Fpr
e

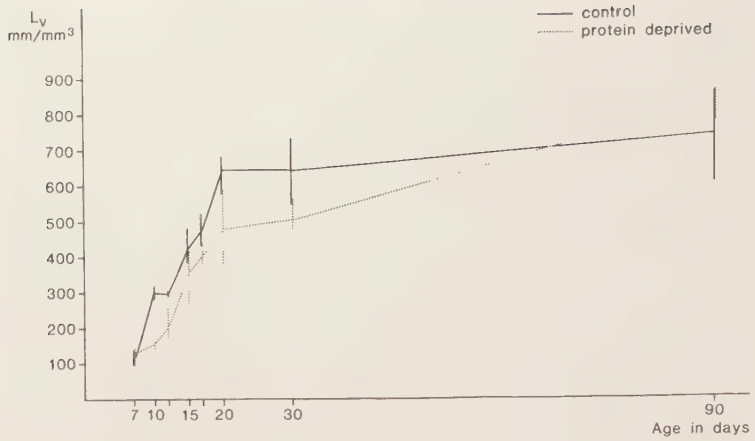


Fig. 6. The specific length of vessels Lv (mm/mm³) in rat neocortical area 18 during postnatal development (days). Vertical lines represent total interindividual variation.

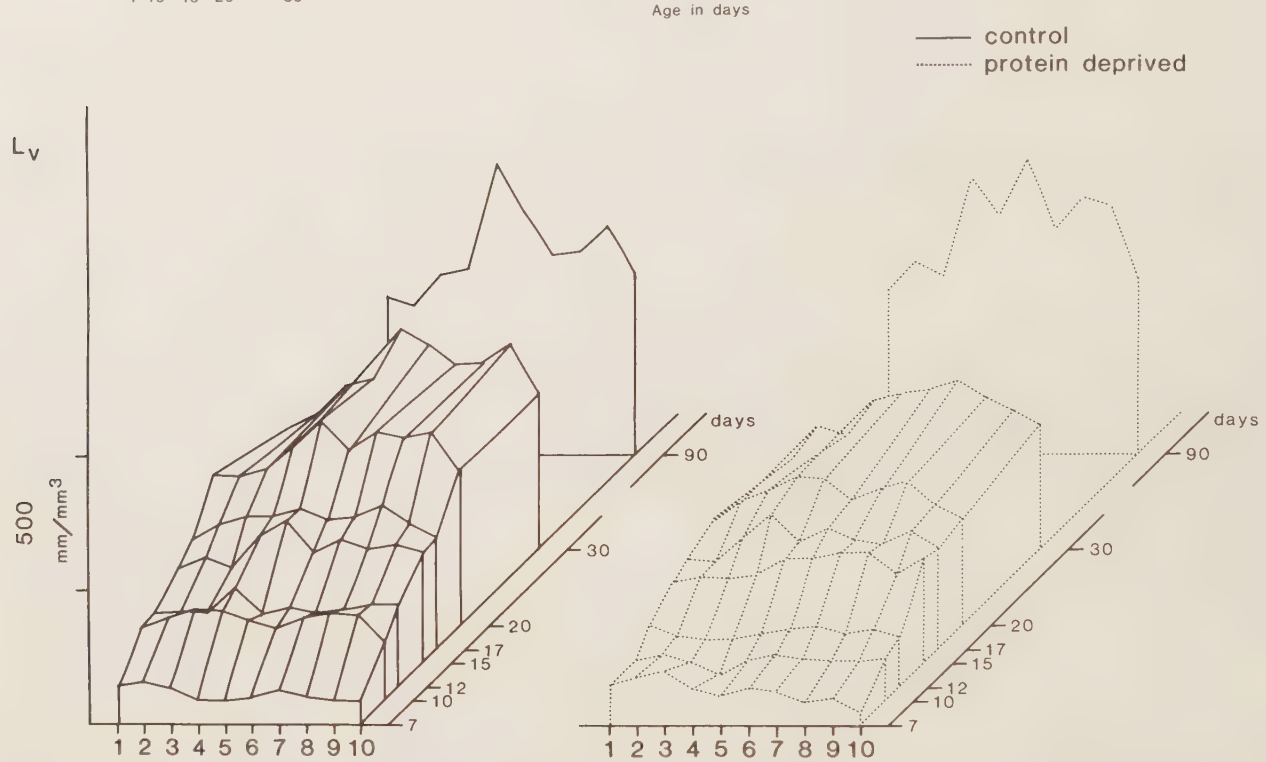


Fig. 7. Cortical distribution (1–10) of the specific length of vessels Lv (mm/mm³) during postnatal development (days). One (1) to 10 represents one tenth of the cortical thickness, 1 being the most superficial part.

Growth of capillaries in the cerebral cortex of normal rats (paper III)

During the postnatal development the Lv of area 18 increased rapidly from a mean of 110 mm/mm³ in 7 day old rats to 480 mm/mm³ in 17 day old rats (Fig. 6). The increase was approximately the same in the ten levels of the cortex (1 – 10, Fig. 7). There was a distinct peak of the Lv in levels 4, 5 and 6 in 20 day old rats corresponding to the Lamina IV of the cortex. The highest Lv in this peak (level 5) was 758 mm/mm³ (range: 704 – 804), while the mean for the cortex was 645 mm/mm³ (range 579 – 679). The peak of the Lv in Lamina IV was even more marked in 30 and 90 days old rats. A small peak was formed in level 9 also.

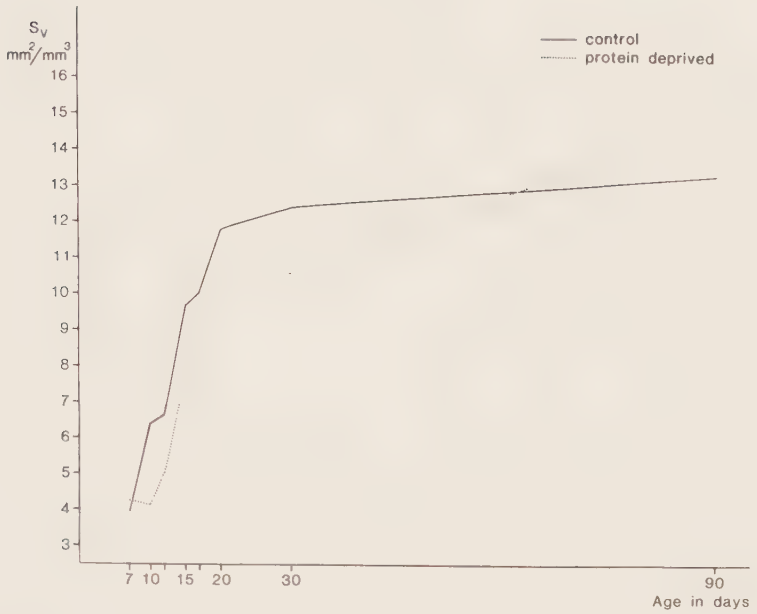


Fig. 8. The specific surface of vessels Sv (mm^2 /mm^3) in rat neocortical area 18 during postnatal development (days).

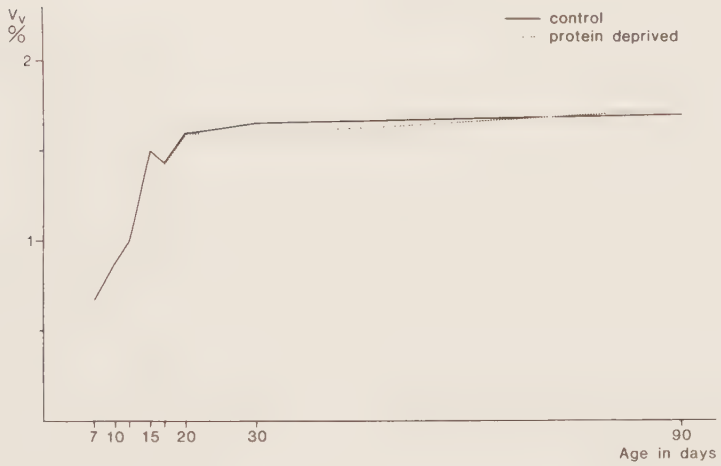


Fig. 9. The volume fraction of vessels Vv (%) in rat neocortical area 18 during postnatal development (days).

The changes in the Sv and the Vv during development corresponded to those of the Lv. The Sv increased from 3.9 mm^2 /mm^3 in 7 day old to 11.8 mm^2 /mm^3 in 20 day old rats (Fig. 8). During the same period the Vv increased from 0.7 % to 1.6 % (Fig. 9). Histograms of the lumina of vessels showed that the majority of the vessels were in the capillary range ($\emptyset < 8.25$ μm, *cf.* Bär & Wolff 1973) at all ages studied (Fig. 10). Most vessels belonged to the 6.0 and 7.5 μm fractions in rats 7 days old. From 10 days of age most vessels belonged to the 6.0 and 4.5 μm fractions. The mean diameter of lumina of capillaries decreased from 5.3 μm in rats 7 days old to 4.5 μm in rats 20 days old.

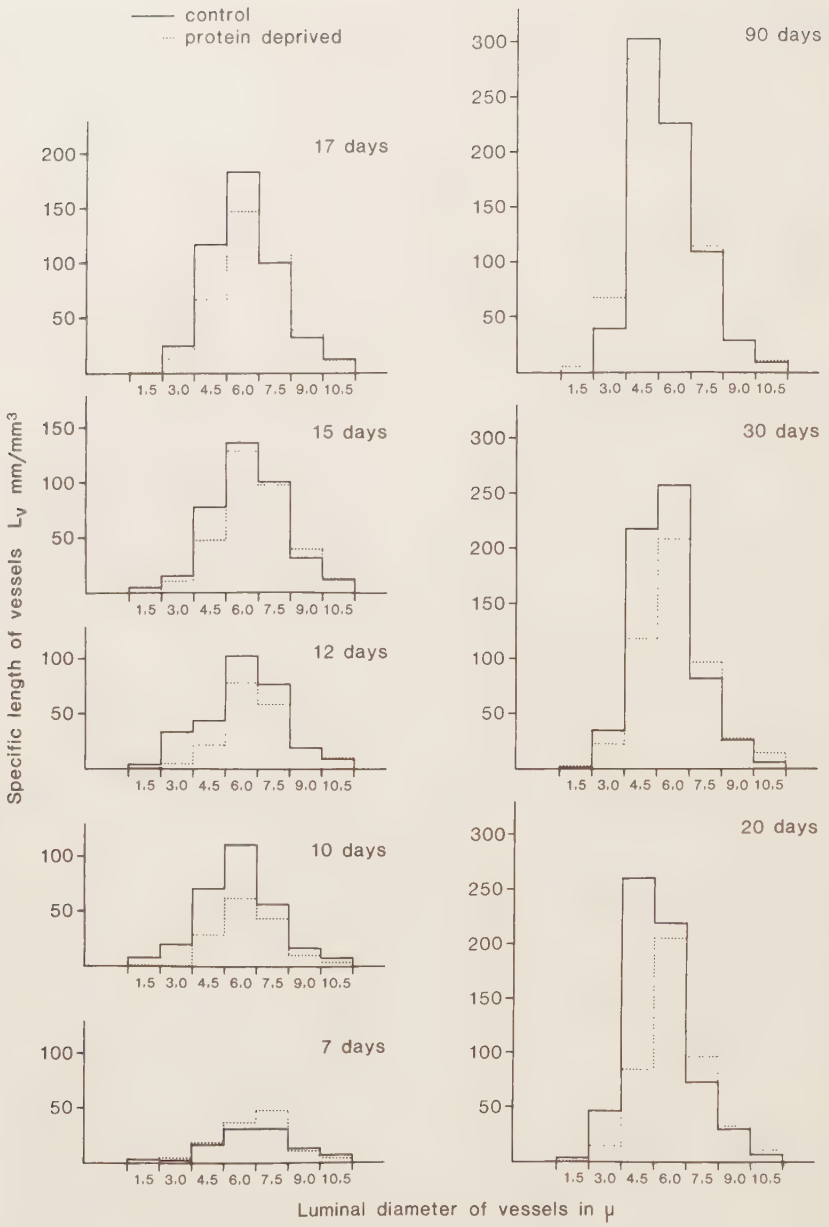


Fig. 10. Size distribution of luminal diameters expressed as specific length of vessels for each fraction at the ages examined (days). Figures on horizontal lines give the mid-point of each fraction in μ (o.75-1.5-2.25).

Growth of capillaries in the cerebral cortex of protein deprived rats (paper III)

No difference in the Lv was seen between normal and protein deprived rats 7 days old (Fig. 6). From 7 to 10 days the Lv of protein deprived rats increased by some 30 mm/mm³ compared with an increase of 190 mm/mm³ in the normal rats. There was a significant difference between the normal and the protein deprived rats from 10 days. This difference was fairly constant up to 30 days due to the comparable rates of increase in the two groups. The distribution of the Lv of the protein deprived rats over the cortex was uniform during the early prenatal development (Fig. 7). No distinct peak in levels 4, 5 and 6 (Lamina IV) was seen in the cortex of the protein deprived rats at 20 or 30 days. The distribution of vessels in the cortex and the mean Lv of the cortex in the

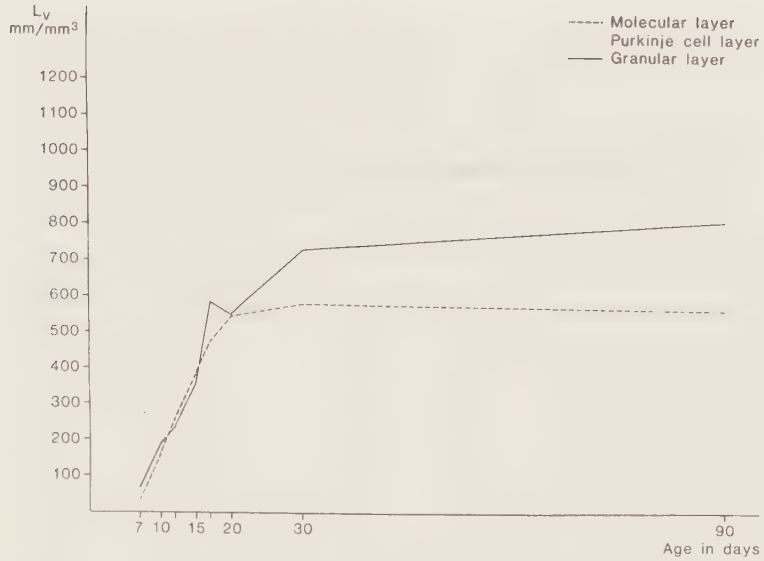


Fig. 11. The specific length of vessels Lv (mm/mm³) in the molecular, Purkinje cell and granular layers of normal rats (Lobule IX) during postnatal development (days).

protein deprived rats 90 days of age were not different from that of 90 day old normal rats. Only slight differences were seen between the normal and the protein deprived rats in the Sv and Vv of the cortex (Fig. 8, 9). There was no major difference in the histograms of the diameters of lumina between the two groups (Fig. 10). The mean diameter of lumina of capillaries seemed to be slightly higher in the protein deprived rats up to 30 days. The mean diameter of lumina of capillaries, the Sv and the Vv of the protein deprived rats were not significantly different from that of the normal rats. This may be due to the relatively large variation between individuals in these variables.

Growth of capillaries in the cerebellar cortex of normal rats (papers II, IV)

The Lv of the cerebellar cortex of normal rats demonstrated a markedly higher Lv in the Purkinje cell layer (200 mm/mm³) than in the granular (65 mm/mm³) and molecular (35 mm/mm³) layers of rats 7 days old (Fig. 11). The Lv of the Purkinje cell layer increased rapidly to a mean of 805 mm/mm³ in rats 20 days of age and by a subsequent lower rate to 980 mm/mm³ in rats 90 days of age. Most vessels in the Purkinje cell layer of young rats were in the capillary range. The Lv of the granular layer was 550 and 810 mm/mm³ at 20 and 90 days while the Lv of the molecular layer was 550 and 565 mm/mm³ (Fig. 11). The developmental increase in the Lv was thus approximately the same in the molecular and the granular layers during the first three weeks after birth. Some difference was seen between the Lv of these layers in rats 90 days old. The Lv of the Purkinje cell layer was clearly higher than that of the molecular layer at all ages (see also below).

The morphometrical results (IV) were corroborated by the study on ink-injected material (II). Thin vessels were seen to be aligned to the Purkinje cell layer of the archi- and paleo-cerebellar parts in rats 1 day old (Fig. 12). Vessels which were aligned to the Purkinje cell layer were numerous in the archi- and paleo-cerebellar parts in rats 5 days old. Only occasional vessels aligned to the Purkinje cell layer were seen at the depths of sulci at this age in the neo-cerebellar parts. In rats 10 days old a capillary plexus of varying density was present in all lobules of the vermis of the cerebellum (Fig. 13). The density of capillaries of the Purkinje cell layer was higher than that in the molecular and inner granular layer (Fig. 13).

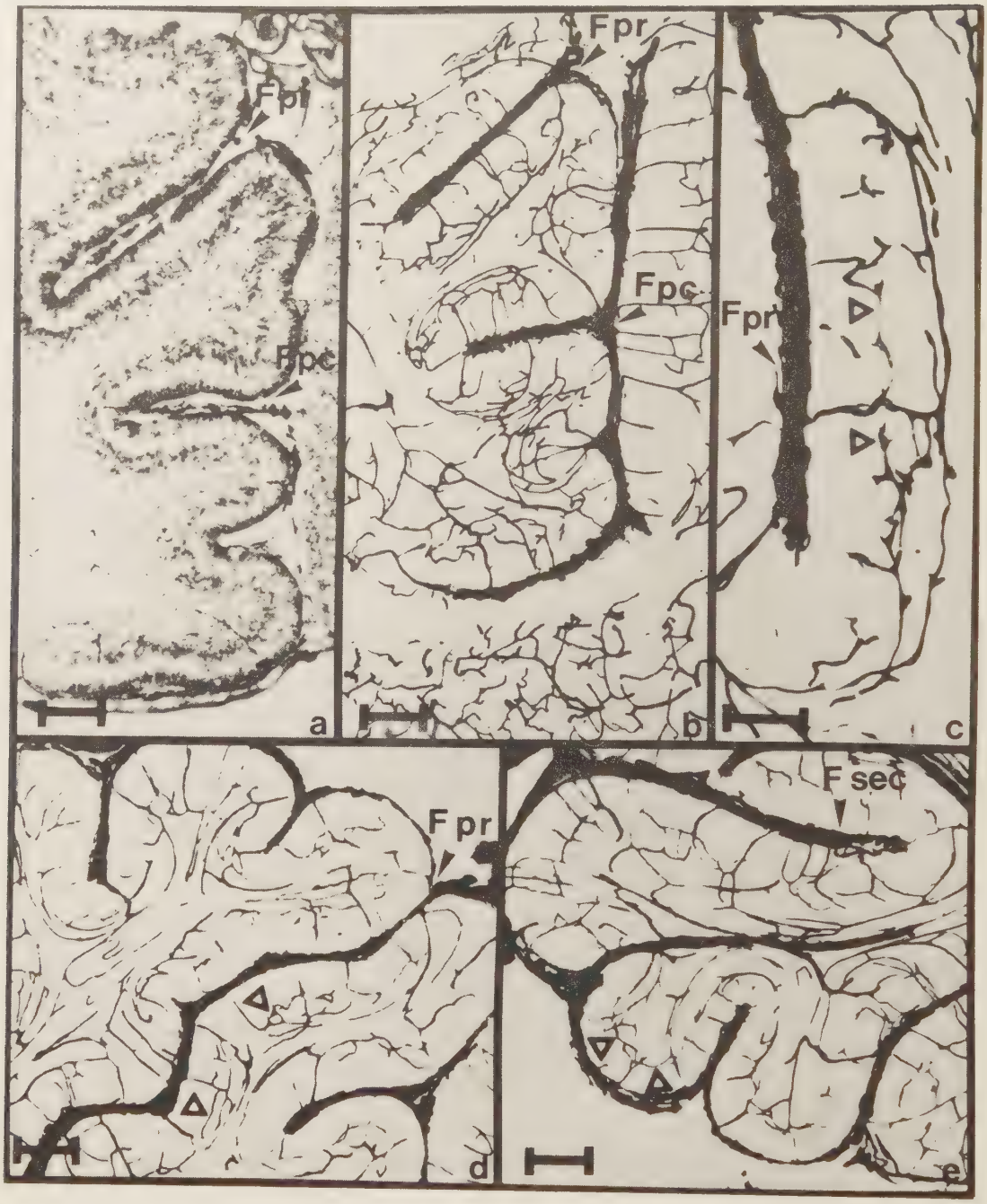


Fig. 12 ***a****. Parasagittal section through the cerebellar vermis of a 1-day-old rat stained with toluidine blue. Note the cortical layers and early fissures (F.pr. = Primary fissure; F.pc. = Preculminate fissure; Bar = 200 µm).* ***b****. Parasagittal section through the cerebellar vermis of a 1-day-old rat. Note vascular plexus in the Purkinje cell – Granular layer. (cf.* ***a****, Bar = 200 µm).* ***c****. Parasagittal section through the cerebellar vermis of a 1-day-old rat. Note the alignment of some loops to the Purkinje cell layer (arrows) (cf.* ***a*** *and* ***b****). (F.pr. = Primary fissure; Bar = 100 µm).* ***d****. Parasagittal section through the anterior vermis of a 5-day-old rat. Note the capillary loops in the Purkinje cell layer (arrows) in the archicerebellar parts but not in the neocerebellar parts (upper left). (F.pr = Primary fissure; Bar = 200 µm).* ***e****. Same as in* ***d****, posterior parts. (F.sec. = Secondary fissure; Bar = 200 µm).*

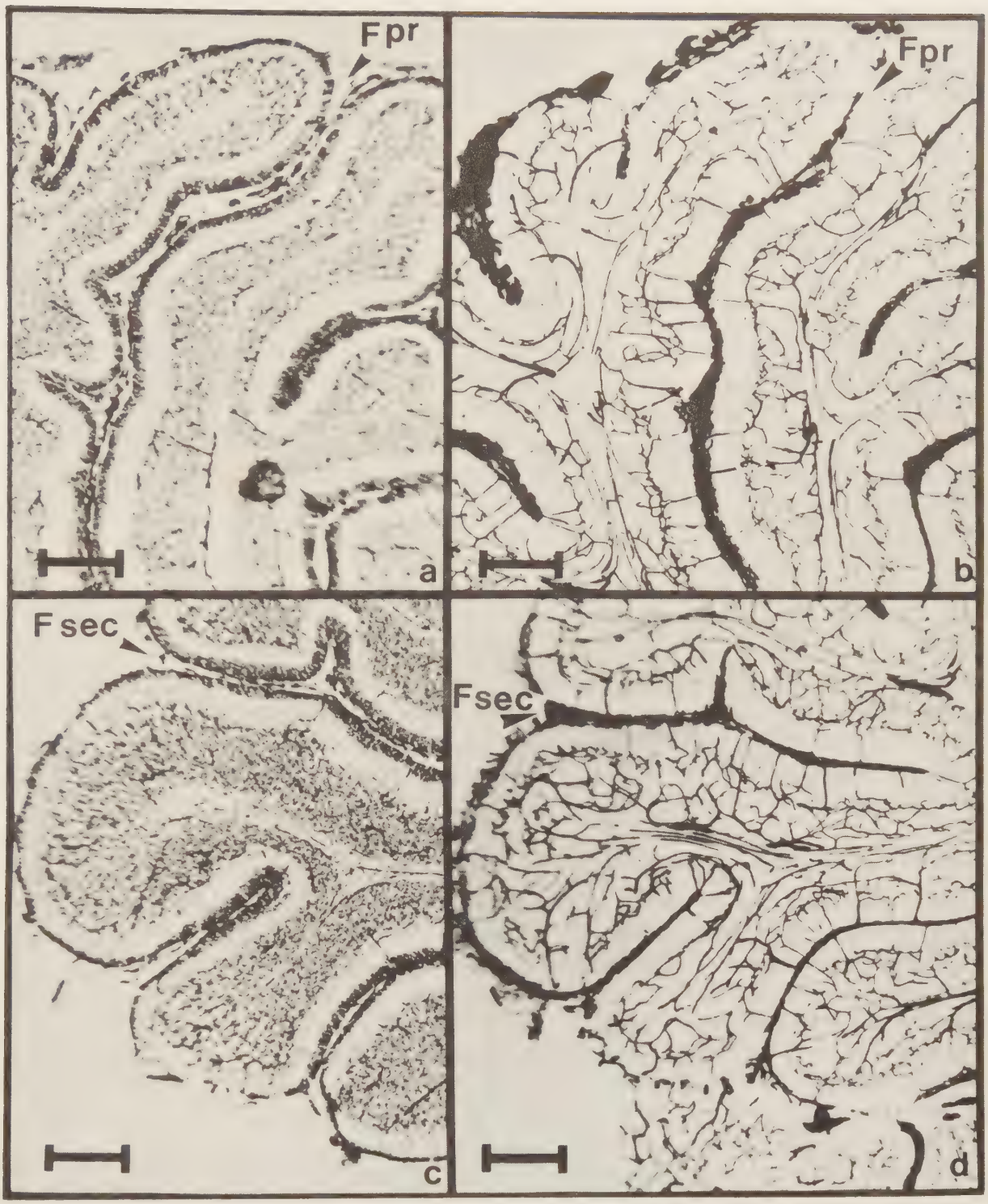


Fig. 13 ***a–d****. Parasagittal sections through the vermis of a 10-day-old rat.* ***a****. Section 20 μm thick stained with toluidine blue. Note the cortical layers.* ***b****. Same area as in* ***a****. Note the capillary plexus in the Purkinje cell layer of all lobules.* ***c****. Section 20 μm thick stained with toluidine blue. Note the cortical layers.* ***d****. Same area as in* ***c****. Note the capillary plexus in the Purkinje cell layer of all lobules. (F.pr. = Primary fissure; F.sec. = Secondary fissure; Bars = 400 μm).*

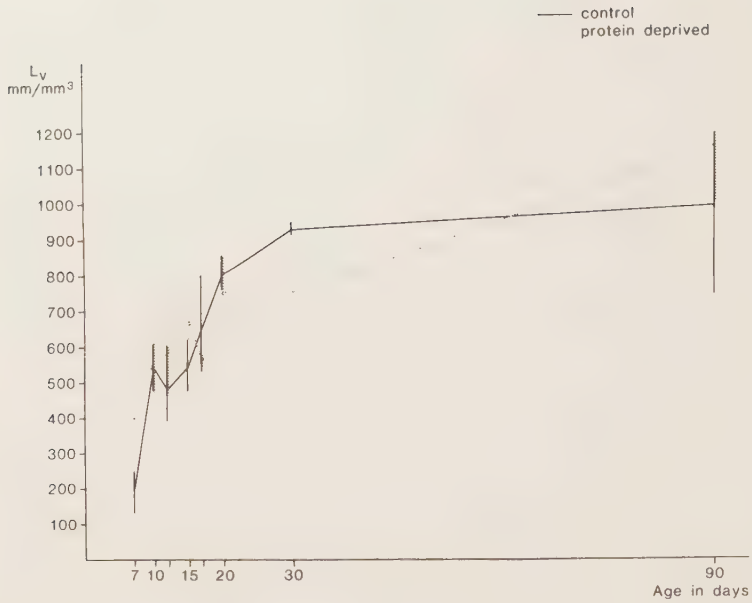


Fig. 14. The specific length of vessels Lv (mm/mm³) in the Purkinje cell layer of lobule IX during postnatal development (days). Vertical lines show total interindividual variation.

Apart from the stem vessels only occasional vessels were seen in the molecular layer before 10 days of age. The formation of vessels in this layer proceeded from the Purkinje cell layer towards the surface of the cerebellum. No differences were seen between deep and superficial parts of the granular layer. The plexus of vessels was present from the early postnatal period in the granular layer. This plexus became successively denser with age (Fig. 12, 13).

Growth of capillaries in the cerebellar cortex of protein deprived rats (paper IV)

The Lv of the Purkinje cell layer in protein deprived rats 7 days of age was higher than that in normal rats (390 compared with 200 mm/mm³) (*Fig. 14*). The Lv of the Purkinje cell layer continued to increase after 10 days in protein deprived rats and was significantly increased at 15 days compared with normal rats. After 15 days there was a low rate of increase in protein deprived rats but, although the Lv of the Purkinje cell layer in protein deprived rats appeared to be reduced at 30 days compared with controls the difference was not significant. The Lv of the Purkinje cell layer was markedly higher than that of the molecular layer in the protein deprived rats. The differences of the mean Lv between the Purkinje cell and the granular layer were approximately the same at 30 and 90 days of age in both groups. The difference in the Lv between these two layers, tested for all animals 30 and 90 days of age, was significant and the findings thus indicate a higher Lv in the Purkinje cell layer than in the granular layer in adult rats.

The rate of increase of the Lv in the granular and the molecular layers of the protein deprived rats from 7 to 10 days of age was low. The protein deprived rats were significantly different from the normal rats when the differences were tested for the interval 7 to 20 days of age. Only slight differences were seen between the protein deprived and normal rats with regard to the Lv in the granular and molecular layers after 20 days of age (Fig. 15, 16).

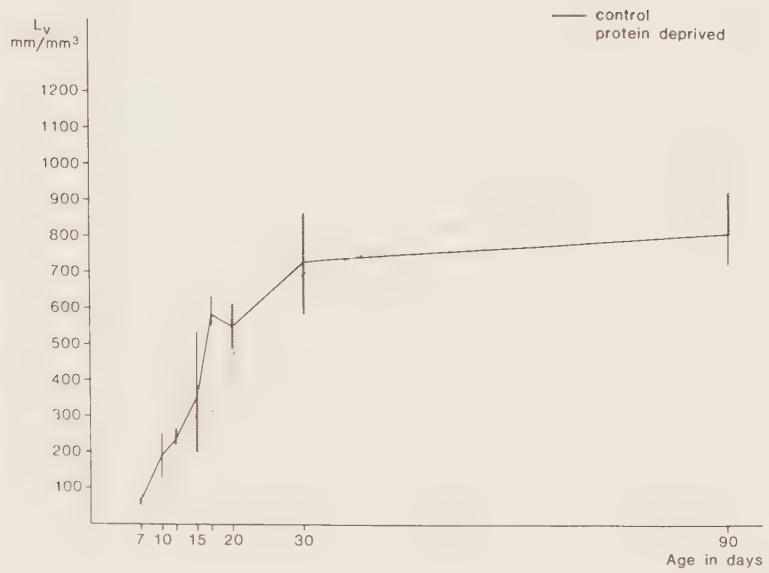


Fig. 15. The specific length of vessels Lv (mm/mm³) in the granular layer during postnatal development (days). Vertical lines show total interindividual variation.

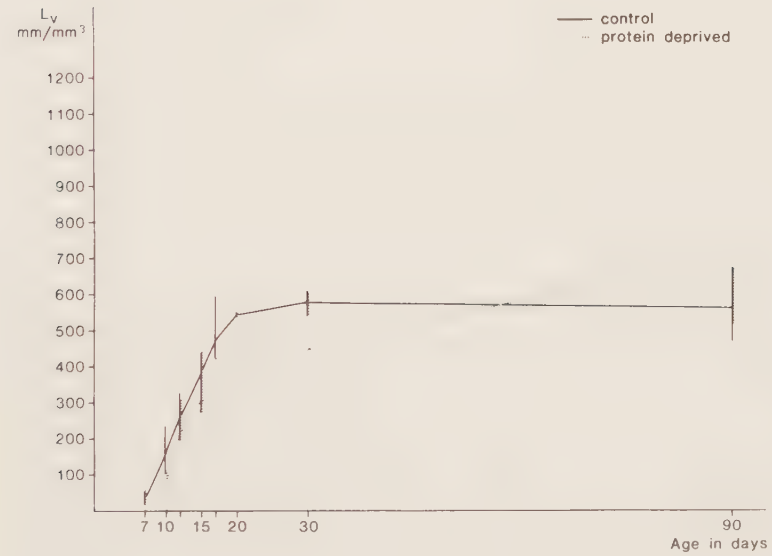


Fig. 16. The specific length of vessels Lv (mm/mm³) in the molecular layer of lobule IX during postnatal development (days). Vertical lines show total interindividual variation.

Formation and branching of stem vessels (papers I, II)

The first stem vessels to enter the hindbrain and the spinal cord of the chick show distinct localizations (Williams 1937, Feeney & Watterson 1946). Williams (1937) suggested that the gradients of the formation of stem vessels were due to differences in the metabolic activity of different regions during development. Feeney and Watterson (1946) regarded metabolic factors as too unspecific to explain the high number of stem vessels formed at the border between the alar and basal plates in the spinal cord of the chick during early embryonic development. The formation of stemvessels in the telencephalon of the embryonic rabbit shows distinct temporo-spatial gradients but the density of stem vessels is approximately the same in all regions (Strong 1964). The formation of stem vessels which terminate in the neo-cortex of the rat follows to a major extent the tangential and horizontal expansion of the cortex (Wolff 1978, Bär 1980).

The present study illustrates two different patterns of formation of stemvessels. The stem vessels in the embryonic brain appeared to form in parallel to the expansion of the surface and the thickness of the wall. No differences in the density of stem vessels were seen in different regions. Numerous stem vessels were seen at the depths of fissures in the cerebellum and their formation parallelled the formation of fissures. This finding, and that of Feeney and Watterson (1946) indicate that the formation of stem vessels in the brain and spinal cord is not only a function of the area of the surface or the thickness of *e.g.* the cortex but that it is also controlled by factors within the nervous tissue.

The branching and termination of stem vessels both in the embryonic brain and in the cerebellum was first seen in deep and later in successively more superficial levels. This is in agreement with the view that the level of termination of a stem vessel is an indication of its time of formation (Wolff 1978).

The first set of stem vessels in the embryonic brain was always present in these regions where a plexus of vessels was seen in the ventricular zone. This is consistant with the view that the earliest plexus consists of branches of stem vessels. Paired vessels which would indicate the existence of hair-pin loops were never seen in the cerebrum or the cerebellum during development.

The first set of stem vessels was seen always in regions where the marginal zone had been formed. A temporal and spatial correlation between the formation of the first stem vessels and the marginal zone was also noted by Strong (1964). The appearance of vessels in the embryonic brain thus coincides with the formation of the first neurons and glioblasts. The stem vessels of the first set branched in the ventricular zone and formed a plexus close to the ventricles. The termination of these stem vessels thus appears to be related to the proliferation of the ventricular cells. Some sprouts from stem vessels of the first set penetrate the deepest parts of ventricular zone and make contacts with the ventricles (Williams 1937, Klossovskii 1963). These sprouts which are directed too much towards the ventricles will not be able to establish contacts with other segments of vessels and remain as sprouts. The function of such endothelial ventricular contacts is not known. Sprouts of vessels appear rarely to be directed towards the surface of the brain both in the cerebral (Wolff 1978, Bär 1980) and in the cerebellar cortex since superficial parts receive vessels

mostly from the sides and from the superficial regions. The localization of the plexus formed by the first stem vessels may thus be the result of the probability for a sprout to establish contact with an adjacent vessel.

The endothelial lining of the vessels in the brain during early embryonic development does not restrict, at least not totally, the passage of macromolecules from the blood into the nervous tissue. If the extra-vasated macromolecules and water can reach the ventricles then the earliest vessels may participate in the formation of spinal fluid at these early stages when no choroid plexus has been formed. The pressure of the spinal fluid within the ventricles is necessary for the expansion of the brain (Desmond & Jacobson 1977).

A second set of stem vessels was seen in regions where the intermediate zone had been formed. The intermediate zone consists of immature neurons which, at least during later stages migrate along radial glial fibres some of which are connected to the outer surface of the brain (see also below). These radial glial fibres may form a route for the passage of information from the deeper parts of the embryonic brain to its surface. The formation of the second set of stem vessels may be related to the formation of neurons or radial glial fibres or to the expansion of the surface of the brain.

The branches of the stem vessels of the second set formed a plexus of vessels in the superficial parts of the ventricular zone. The two plexuses thus present in the ventricular zone are confluent and probably correspond to the double-tiered plexus described by Strong (1964) in the telencephalon of the embryonic rabbit. Although it is difficult to compare the results of the present study with those of earlier reports, partly due to differences in terminology, similar observations were probably made by Williams (1937), Feeney and Watterson (1946) and Klossovskii (1963).

Statistical evaluation has corroborated the hypothesis that the blood vessels influence the formation of glioblasts in the ventricular zone (Rickmann & Wolff 1976). The hypothesis was based on the following observations (for ref. see Review). Some mitotic spindles have a cleavage plane which is more or less parallel to the surface of the ventricles. Cells showing such mitotic spindles may retain the outer cytoplasmic process during the mitosis. The cytoplasmic process may contact either the outer surface or vessels in the nervous tissue. The outer daughter cell formed by the mitosis may loose its contacts at the ventricular surface but retain the outer contact. The cell thus formed would show one characteristic feature of astrocytes, namely contact with blood vessels. The hypothesis gains further support by the observation that radial glial cells which loose their contacts at the ventricular surface and which are connected to blood vessels are transformed into astrocytes (Smechel & Rakic 1978).

The plexus of vessels in the superficial parts of the ventricular zone may present a surface with which the outer processes of the ventricular cells can establish contact during the period of migration of the nucleus and of synthesis of DNA. This plexus of vessels may shorten the distance which the outer process has to grow and thus influence the proliferation rate of the ventricular cells and the type of cells to be formed.

The two plexuses of vessels in the ventricular zone may thus possibly differ in function and morphogenetic relations to the surrounding ventricular cells and this justifies the application of different names to them. They were named, according to their localization as the deep and superficial plexus of the ventricular zone.

Stem vessels were formed also during subsequent development. These vessels terminated in the subventricular/intermediate zones. The branches of stem vessels lying outside the ventricular zone may of course also influence the formation of glioblasts from ventricular cells and the formation of astrocytes from radial glial cells as described previously. Migrating neurons follow the radial glial fibres towards the outer surface. The radial glial fibres are connected either to the outer surface of the brain or to the blood vessels within the brain (Smechel & Rakic 1978). This presents the possibility that the position of blood vessels may influence the migration of neurons as was recently suggested for some, later non-pyramidal neurons (Wolff *et al.* 1978). These findings may be related to the view of Dorello (*cit.* Pfeifer 1928) that the formation of a nucleus of nerve cells is preceeded by the formation of the corresponding vascular plexus.

The formation of stem vessels in the brain during the period embryonic day (E) 12 to 15 was not influenced by maternal protein deprivation. There was also a normal timing of the appearance of zones in the brain during this period. Earlier studies on PCM have demonstrated a reduced DNA-content of the brain on embryonic day (E) 16 while the DNA-content of the placenta was reduced already at E 13 (Winick 1970a). The findings of the present study indicate that vessels neither add to the restriction of nutrients nor affect the morphogenesis of the brain in protein deprived rats during early embryonic development.

More stem vessels were formed at the depths of fissures than at other parts of the surface in the normal cerebellum. A similar pattern was also described by Feeney and Watterson (1946) in the cerebellum of the embryonic chick. The thickness of the external granular layer increases locally prior to the formation of the fissures (Larsell 1952). A higher mitotic rate of the cells of the external granular layer at the depths of fissures than of those in the crests of the lobules has been described (Mares & Lodin 1970). The higher number of stem vessels formed at the fissures may be explained by local differences in the external granular layer. Heterotopic cells which resemble the cells of the external granular layer have been found to be located mainly outside vessels in the cerebellum (Pfaffenroth & Das 1974). The cells of the external granular layer originate in the germinal trigone and spread over the surface of the cerebellum. The observations on the heterotopic cells indicate that the migration of the cells in the external granular layer may be influenced or directed along blood vessels. A local increase in the density of stem vessels could result in an increased thickness of the external granular layer. Of course, the formation of stem vessels and the proliferation rate of the cells in the external granular layer can be controlled by some other structural or chemical factor in the cerebellum.

The distance between the depths of the early fissures changes very little during development (Haddara & Nooreddin 1966). The cerebellum thus expands merely by elongation of the lobules which deepen the fissures. The vessels entering at the early fissures will be forced to elongate in the pial plexus to cover the surface of the lobules. This mechanism may explain the parallel arrangement of vessels in the pial plexus noted also by Pfeifer (1928). The formation of numerous stem vessels at the early shallow fissures may be a prerequisite for the adequate formation of stem vessels during subsequent development in these parts of the cerebellum which face the fissures.

Some stem vessels terminated with a strong spatial relation to the Purkinje cells. This occurred mostly during the first postnatal week during which period the Purkinje cells form transitional synapses with climbing fibres and become rich in mitochondria (Altman 1972). The stem vessels which terminated in the molecular layer did so concommitant with the deposition of basket and

stellate neurons and formation of synapses in this layer (Altman 1972). The termination of stem vessels in the cerebellum during postnatal development thus appears to be correlated with the differentiation of neurons as is also the growth of capillaries (see below).

Growth of capillaries in the brain (papers II, III, IV)

The present results on the growth of capillaries in the neo-cortex are in agreement with earlier reports (Craigie 1938, Gyllensten 1959a, b, Bär & Wolff 1973). The rapid growth of capillaries coincides with the differentiation of neurons both in respect of the formation of synapses and changes in metabolism. The number of astrocytes in the neo-cortex increases also during this period. A higher specific length of vessels was seen in Lamina IV than in the rest of the cortex from 20 days postnatally and appears to be related to the maturation of the thalamo-cortical projection system and the corresponding increase in the activity of the oxidative metabolism (*cf.* Friede 1966, Chow & Leiman 1970).

Two major differences were seen with regard to the growth of capillaries in the neo-cortex between normal and protein deprived rats. Firstly the rapid increase in the length of vessels per volume was delayed by some three days in the protein deprived rats. This delay may be correlated to an impaired differentiation of neurons *e.g.* a decreased density of synapses and a reduced activity of Na^+/K^+-ATPase per mg protein in the synaptosomal fraction (Dyson & Jones 1976a, b, Kissane & Hawrylewicz 1975). The developmental changes in oxidative metabolism appear to be less influenced by PCM in relation to growth and net deposition of protein in the brain than is the formation of synapses (for ref. see Review). Secondly, no distinct peak was seen in the specific length of vessels in Lamina IV even at 30 days postnatally in the protein deprived rats. This difference may be correlated to increased latencies in the visual evoked potentials in protein deprived rats at 20 days postnatally (Morgane *et al.* 1978). Preliminary observations indicate alterations in the visual evoked response also after 20 days in protein deprived rats (Andersson, Sjöström & Conradi). The findings indicate that the maturation of the thalamo-cortical projection system is altered by early PCM. We are at present investigating whether there is a corresponding delay of the normal increase in activity of enzymes involved in the oxidative metabolism in Lamina IV.

The growth of capillaries in the cerebellum of the rat has previously been examined by Craigie (1924), who found an increase in the length of vessels per volume from day 10 postnatally in the granular and molecular layers but does not state which region was studied. The present study has demonstrated the formation of a capillary plexus in the Purkinje cell layer of all lobules during the early postnatal development in the rat. The Purkinje cells have somatic spines forming transitional synapses with climbing fibres and form apical cones rich in mitochondria during this period. The Golgi epithelial cells (Bergman glia), the somas of which lie close to the Purkinje cells increase in number and differentiate during this period or somewhat later (Das 1976). The differentiation of the Purkinje cells is known to take place earlier in the archi- and paleo-cerebellum and at the depths of fissures than in the neo-cerebellum and in the crests of lobules (Altman 1972). The same gradients were seen for the growth of the capillaries in the Purkinje cell layer. There seemed to be no or only a slight increase in the specific length of vessels in the Purkinje cell layer between days 10 and 15 postnatally and this may be correlated to the disappearance of the somatic spines and the outgrowth of the primary dendrites of the Purkinje cells with a concomitant decrease in the activity of the oxidative metabolism in the Purkinje cell soma (Altman 1972). The increase in

the specific length of vessels in the Purkinje cell layer between 15 and 30 days may be related to the concomitant formation of synaptic contacts between Purkinje cells and basket cell terminals (Altman 1972) accompanied by an increase in the activity of oxidative enzymes in the Purkinje cell soma (Persson & Sima 1975).

The present findings have indicated a higher specific length of vessels in the Purkinje cell layer also in adult rats. Conighi (1922) claimed that there was a special capillary net surrounding the Purkinje cells but this has been denied by several authors (*e.g.* Fazzari 1924, Uchimura 1929, Craigie 1938). Since the capillaries in the Purkinje cell, granular and molecular layers are confluent it may be inappropriate to separate them. The differences may, however, have functional and morphogenetic consequences especially during the early prenatal development when the differences appear to be relatively larger than in adult rats.

The growth of capillaries in the molecular layer followed the same gradients as did that in the Purkinje cell layer. The formation of capillaries proceeded from the deep to the superficial parts thus parallelling the outgrowth of the Purkinje cell dendrites and the increased oxidative metabolism in this layer (Altman 1972). The specific length of vessels increased rapidly both in the molecular and granular layers from day 7 postnatally. The plexus of capillaries in the granular layer became successively denser with age showing no distinct gradient in any lobule but regular differences between the lobules. This is in agreement with findings on the differentiation of the neurons in this layer (Altman 1972).

The specific length of vessels in the Purkinje cell layer at 7 days was higher in protein deprived than in normal rats. Persson and Sima (1975) found no differences in the activity of several enzymes involved in oxidative metabolism in normal and PCM rats during the first week. There may be an increased packing density of the Purkinje cells in PCM rats (Neville & Chase 1971). The density of climbing fibre synapses in the Purkinje cell layer has not been examined after PCM. The growth of vessels per volume in the Purkinje cell layer took place at a higher rate in the protein deprived rats between 10 and 15 days of age possibly related to a prolonged persistence of somatic spines and delayed outgrowth of the primary dendrites of Purkinje cells of PCM rats (Sima & Persson 1975).

The rapid growth of capillaries was delayed in the molecular and granular layers of protein deprived rats, there being a delay of approximately three days compared to the normal rats. No difference in the specific length of vessels in the granular layer was seen between normal and protein deprived rats at 20 or 30 days postnatally. No gross alterations of the activity of oxidative enzymes have been demonstrated in the granular layer after early PCM (Persson & Sima 1975). The formation of granule cells is prolonged and the packing density of these cells increased by PCM. The specific length of vessels in the molecular layer of protein deprived rats was reduced also at 20 and 30 days postnatally. The values for the specific length of vessels in this layer may be slightly influenced by vessels which surround the Purkinje cells. Persson and Sima (1975) found no differences in the activity of oxidative enzymes in this layer between normal and PCM rats. A reduced density of synapses in this layer has been demonstrated after early PCM and the growth of the Purkinje cell dendrites is also affected.

The effects of PCM and of hypothyroidism on the development of the brain differ in some respects (*e.g.* Eayrs & Horn 1955, Clos *et al.* 1973, Dainat *et al.* 1974) but both appear to negatively influence the differentiation of neurons (*c.f.* Review, Legrand 1977). Eayrs (1954) described

a decreased number per area but increased size of vascular fragments in the brain of hypothyroid rats. These findings when compared with those of the present study indicate that the mechanisms behind altered growth of vessels in the brain are similar in PCM and hypothyroid rats.

The control of the growth of capillaries in the brain

The mitotic division of the endothelial cell may not only give rise to cells, which contribute to the elongation and dilatation of vessels but is also involved in the formation of sprouts (Klossovskii 1963, Bär 1980). Redistribution and elongation of the endothelial cells and a decrease in the length of the inter-endothelial contacts can also result in elongation and dilatation of vessels (Bär 1980). Several studies have indicated that the growth of blood vessels during development is related to the differentiation of neurons, two major aspects of which have been considered; the formation of synapses and the increased activity of the oxidative metabolism and number of mitochondria (for ref. see Review). Some changes in the oxidative metabolism during development are probably related to the formation of synapses. The development of astrocytes is also related to the differentiation of neurons and must be considered when attempting to explain the control of the growth of vessels in the brain.

The specific length of vessels in the neo-cortex increases after prolonged hypoxia (Bär 1980) and decreases after breeding of the animals in a high oxygen tension during early development (Gyllensten 1959b). These findings indicate that the growth of vessels may be controlled by some metabolites of the oxidative metabolism. However both hypoxia and raised oxygen tension may affect the differentiation of neurons in the cortex. Prolonged hypoxia during early development results in an increased proliferation rate of the endothelial cells and an increase in the specific length and the diameter of lumina of the blood vessels without altering the number of branches per volume. It thus appears reasonable to assume that the formation of sprouts is less influenced by factors related to the oxidative metabolism than are elongation and dilatation of the vessels. However, it should be remembered that the dilatation and possibly also the elongation of the vessels may be the result of central circulatory alterations. The discrepancy between the specific length on one hand and the volume fraction of vessels on the other in the neo-cortex of the protein deprived rats during development indicates that the volume of the lumina of vessels was more closely related to the volume of the neo-cortex than was the length of the vessels. There appears to be a corresponding discrepancy between the number of synapses per volume on one hand and the activity of some oxidative enzymes per mg protein on the other in PCM rats. Dilatation of the vessels may be related to metabolic factors in the brain or central circulatory alterations but is insufficient to compensate for the protein deprivation.The reduced specific length of vessels in some regions of protein deprived brains may be correlated to a reduced number per volume and delayed maturation of synapses (for ref. see Review). The findings of Bär and Wolff (1973) indicate that the formation of sprouts is temporally more closely related to the formation of synapses than is the increase in the specific length of vessels. Whether protein deprivation results in a decreased formation of sprouts is, at present, not known but it reduces the proliferation rate of many types of cells (Deo 1978).

The formation of sprouts and the following increased specific length of vessels may be related to any stage in the formation of synapses (Fig. 17). The early increase in the specific length of vessels in the visual cortex appears to be more dependent on intracortical factors than on the afferent input (Gyllensten 1959a). This appears to be the case for the early differentiation of cortical neu-

rons also (*cf.* Wolff 1979a, b). One is tempted to look for a morphogenetic factor which controls both the formation of presynaptic sites and the growth of blood vessels. Such a factor would ascertain a sufficient blood supply when and where synapses are formed and the energy requirements become high. The action of GABA may to some extent explain the formation of postsynaptic sites in both the cerebral and cerebellar cortex. The effects of GABA on the growing vessels in the brain have not been examined. There appears to be, however, a similar delay of the rapid growth of vessels and the increase in activity of enzymes involved in the metabolism of GABA in the protein deprived brain from 7 days postnatally (Rajalakshmi *et al.* 1974).

The maturation of the blood-brain barrier is better correlated to the formation of perivascular astrocytic processes and basement membranes than to any change in the morphology of the endothelial cells. Glial cells induce the formation of gamma-glutamyl transpeptidase in endothelial cells *in vitro* (De Bault & Cancilla 1980). The formation of sprouts decreases when the fraction of the external surface of capillaries covered by astroglial processes increases (Wolff & Bär 1976). These findings indicate that differentiation of endothelial cells may be influenced by astrocytes and result in a decreased rate of growth and formation of branches. Whether astrocytes can induce growth and sprouting of the blood vessels is not known but glioblasts and astrocytes accumulate and release GABA (*cf.* Wolff 1979a, b).

The formation of capillaries in the Purkinje cell layer during the first postnatal week may possibly preceed the formation of Golgi epithelial cells (Das 1976). There may be a corresponding relation between the formation of these cells and the blood vessels as has been suggested recently for both glioblasts in the embryonic brain and astrocytes in the cerebral cortex (Rickmann & Wolff 1976, Smechel & Rakic 1978).

The significance of the growth of vessels in the brain in protein deprivation

The growth, differentiation and function of the brain can be assumed to be dependent on both the constituents in the blood and the transport capacity of the vascular system. The transport capacity of the blood vessels depends on their length, surface and volume and the function of the endothelial cells. The differentiation of endothelial cells in protein deprivation has not hitherto been subjected to any study but we are presently examining the enzymatic content of endothelial cells isolated from the brain. The following discussion will consider only the possible significance of a reduced growth of vessels in the development of the brain.

Protein deprivation *per se* may affect directly the proliferation rate of endothelial cells in the brain as has been shown for many other types of cells (*cf.* Deo 1978). The number of endothelial cells per neuron is decreased in the neo-cortex of PCM rats (Siassi & Siassi 1973). The growth of vessels, including proliferation, elongation and arrangement of endothelial cells appears, however, to be influenced by factors present within the brain. These appear to be related to some, but not all aspects of growth and differentiation of the brain and may also be influenced by the protein deprivation *per se*. This results in three possibilities for the relation between transport capacity of the blood vessels and growth and development of the brain in protein deprivation, namely: the vascularization is a) adequate for the total demand of the brain, b) adequate for this demand which is directly related to the factors which control the growth of the vessels only and c) inadequate

for all aspects of growth and development of the brain. The growth of blood vessels can be regarded to be adequate when the possible negative influences of protein deprivation *per se* on the endothelial cells is overcome by these factors in the brain which stimulate the growth of blood vessels and when the demand of no aspect of the growth and development of the brain is less influenced by the protein deprivation *per se* than the growth of the vessels. These aspects of growth and development of the brain which are only partially related to the factors that control the growth of vessels and which are primarily less influenced by the protein deprivation than the transport capacity of the blood vessels may be significantly affected by the reduced growth of the vessels.

It could be argued that any change in the brain of protein deprived rats is merely caused by the protein deprivation *per se* up to 7 days of age since the vascularization appeared to be un-altered during embryonic development and at 7 days. The vessels cannot, however, at any stage compensate completely for protein deprivation.

The reduction in length of vessels in some regions of the brain in the protein deprived rats after the age of 7 days is without significance if the reduction in length is proportional to a reduction in the demands of the brain. There were indications of an adaptation to the state of reduced length by dilatation of the vessels in the protein deprived rats. The transport capacity of the resulting shorter but wider vascular system in the protein deprived brain may be adequate for all demands of the brain under otherwise normal conditions. There may, however, be a reduced ability of the vessels in the brain of protein deprived rats to respond under other pathological conditions such as hypoxia and hypoglycemia.

Decreased growth of vessels may also have morphogenetic consequences, altering *e.g.* the migration of neurons and formation of astrocytes.

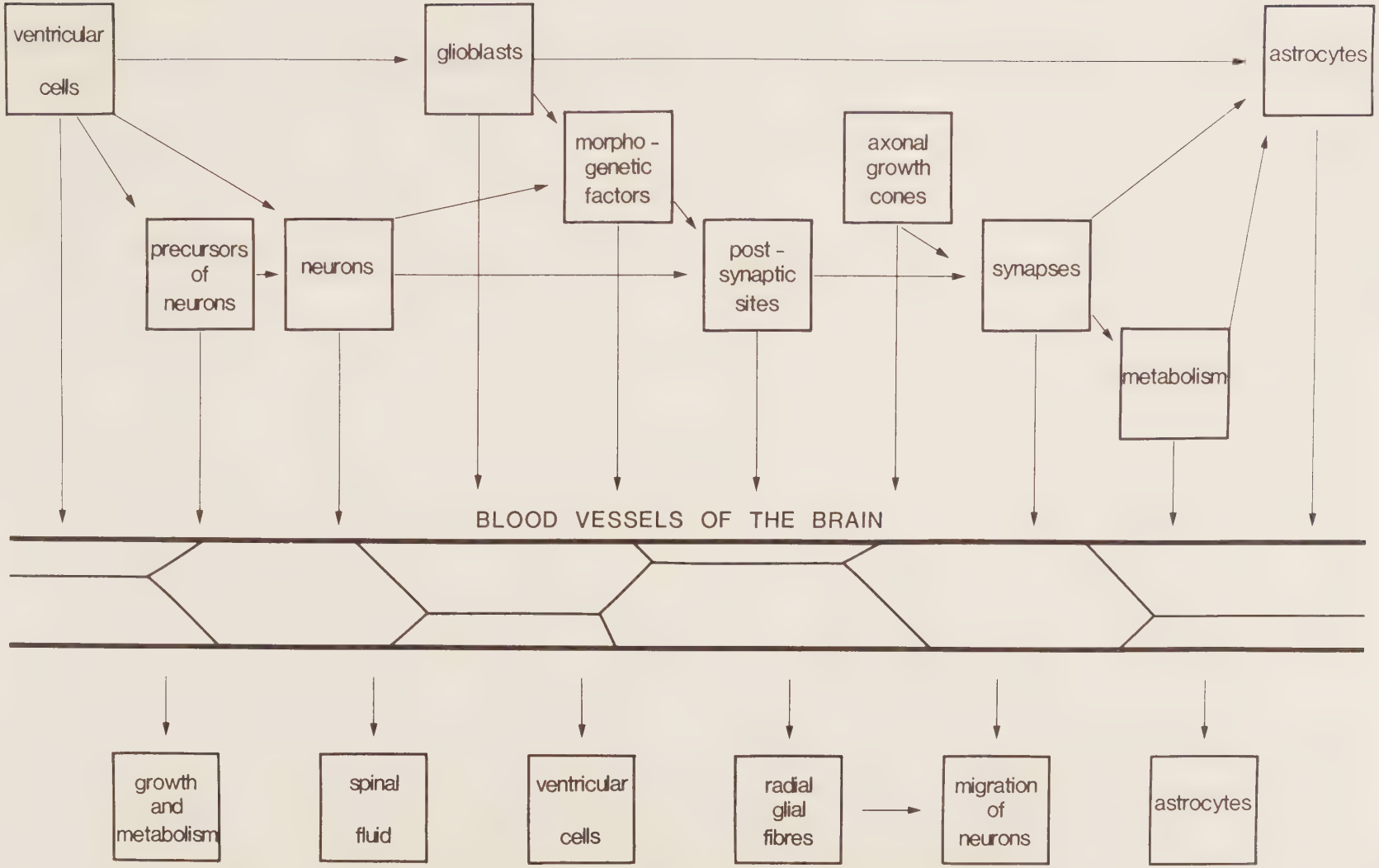
SUMMARY AND CONCLUSIONS

The internal vascularization of the brain is closely related to its growth and development. There is a temporal and spatial correlation between the ingrowth of the first vessels and the appearance of neurons and glioblasts in the embryonic brain. The formation of stem vessels parallels the formation of fissures in the cerebellum and, thus, does not merely follow the expansion of the cerebellar surface but appears to be controlled by factors in the cerebellum. Stem vessels terminate earlier in deep than in superficial levels of the brain; as has been suggested previously by Wolff (1978) the level of termination of a stem vessel appears to be an indication of its time of formation. Branches of stem vessels first give rise to a deep and a superficial plexus in the ventricular zone and, later, to a plexus in the subventricular and intermediate zones. The termination of some stem vessels in the cerebellar cortex shows a close spatial relation to the Purkinje cells. The latest stem vessels in the cerebellum may form and terminate in relation to the differentiation of neurons. The formation and growth of capillaries show distinct temporal and spatial relations to the differentiation of neurons, notably synaptogenesis and increased oxidative metabolism. This is nicely illustrated by the formation of a capillary plexus in the Purkinje cell layer during the early postnatal development in the rat.

Protein deprivation appears not to influence the formation and termination of stem vessels in the embryonic brain during the period embryonic day 12 – 15. There is a normal timing of the appearance of the zones in the embryonic brain during the examined period and earlier findings also indicate that the development of the brain is not affected by protein deprivation before embryonic day 15. The rapid growth of capillaries in the neo-cortex is delayed by some 3 days and the normal increase in the specific length of vessels in neo-cortical Lamina IV is delayed by at least 10 days in protein deprived rats. The specific length of vessels was increased in the Purkinje cell layer in 7 and 15 day old rats and reduced in the granular and molecular layers in 10 – 20 day old protein deprived rats compared with controls. The findings on the growth of capillaries in protein deprived rats correlate well to earlier findings on impaired differentiation of neurons in PCM.

Several possible morphogenetic interactions between blood vessels, neurons and glial cells are discussed and have been summarized in Figure 17. The significance of the growth of vessels in protein deprivation depends on how it is normally controlled. Several studies have demonstrated an altered development of neurons and glia in PCM rats, the vessels thus cannot compensate for the protein deprivation. Even if the transport capacity of the vessels is not reduced to an extent where it will add to the restriction of nutrients during protein deprivation only, there may be a reduced ability of the vessels to respond under other pathological conditions such as hypoxia or hypoglycemia.

Fig. 17. Schematic illustration of some possible morphogenetic interactions between blood vessels, neurons and glial cells in the brain.


ventricular cells
glioblasts
astrocytes
morpho - genetic factors
axonal growth cones
precursors of neurons
neurons
post - synaptic sites
synapses
metabolism
BLOOD VESSELS OF THE BRAIN
growth and metabolism
spinal fluid
ventricular cells
radial glial fibres
migration of neurons
astrocytes

ACKNOWLEDGEMENTS

My sincere thanks are due to:

My tutor in neurobiology, Professor Patrick Sourander, for initiating this study, for continuous support and interest and for providing working facilities.

Professor Joachim-Rudolf Wolff, Göttingen, for his stimulating guidance and cooperation and for making available the indespensible use of the Quantimet,

Dr Siegfried Eins for his never failing enthusiasm in explaining the theory and practice of the Quantimet,

Dr Jan Engvall for stimulating cooperation,

Dr Anders Oldfors for his helpful discussions and suggestions,

Misses Kristina Müntzing, Margareta Persson and Hermengild Schucht for their skilled technical assistance,

Mrs Ingeborg Hartmann for her rapid and elegant typing,

Messrs John Abrahamsson and Hans-Olof Ivarson for the excellency of their photographical work,

Mr Martin Härnquist for his help with statistics,

Dr William Mair for linguistic revision and constructive criticism of this thesis,

Mrs Barbro Johansson for typing the thesis.

Finally, I wish to express my sincere gratitude to my fiancée Dr Åsa Rigéus for her encouragement and interest, which made the writing of the thesis so much easier, and to whom this thesis is dedicated.

This investigation was supported by grants to Professor Patrick Sourander from the Swedish Medical Research Council No. B78–12X–03488–07A and B79–12X 03488–08B, and by grants from the Medical Faculty, University of Göteborg.

REFERENCES

Aby, F.S.: Observations on the blood capillaries in the cerebellar cortex of normal young adult domestic cats. J. Comp. Neurol. 9: 26–33 (1899).

Adlard, B.P.F., Dobbing, J.: Vulnerability of developing brain. III. Development of four enzymes in the brains of normal and undernourished rats. Brain Res. 28: 97–107 (1971).

Adlard, B.P.F., Dobbing, J.: Vulnerability of developing brain. V. Effects of fetal and postnatal undernutrition on regional enzyme activities in three week-old rats. Pediatr. Res. 6: 38–42 (1972a).

Adlard, B.P.F., Dobbing, J.: Vulnerability of developing brain. VIII. Regional acetylcholin esterase activity in the brains of adult rats undernourished in early life. Br. J. Nutr. 28: 139–143 (1972b).

Alsén, S., Petrén, T.: Die Kapillarisierung der Kleinhirnrinde bei Cavia cobaya. Gegenbaurs Morphol. Jahrb. 83: 268–276 (1939).

Altman, J.: Postnatal development of the cerebellar cortex in the rat. I, II, III. J. Comp. Neur. 145: 353–514 (1972).

Altman, J., Bayer, S.A.: Prenatal development of the cerebellar system in the rat. I, II. J. Comp. Neur. 179: 23–76 (1978).

Angulo-Colmenares, A.G., Vaughan, D.W., Hinds, J.W.: Rehabilitation following early malnutrition in the rat: body weight, brain size and cerebral cortex development. Brain Res. 169: 121–138 (1979).

Balazs, R., Dahl, D., Harwood, J.R.: Subcellular distribution of enzymes of glutamate metabolism in rat brain. J. Neurochem. 13: 897–905 (1966).

Banay-Schwartz, M., Giuffrida, A.M., deGuzman, T., Sershen, H., Lajhta, A.: Effect of undernutrition on cerebral protein metabolism. Exp. Neurol. 65: 157–168 (1978).

Barnes, D., Altman, J.: Effects of different schedules of early undernutrition on the preweaning growth of the rat cerebellum. Exp. Neurol. 38: 406–419 (1973).

Barnes, D., Altman, J.: Effects of two levels of gestational-lactational undernutrition on the post weaning growth of the rat cerebellum. Exp. Neurol. 38: 420–428 (1973).

Bass, N.H., Netsky, M.G., Young, E.: Effect of neonatal malnutrition on the developing cerebrum. I. Microchemical and histologic study of cellular differentiation in the rat. Arch. Neurol. 23: 289–302 (1970).

Bär, Th., Wolff, J.R.: The formation of capillary basement membranes during internal vascularization of the rats' cerebral cortex. Z. Zellforsch. 133: 231–248 (1972).

Bär, Th., Wolff, J.R.: Quantitative Beziehungen zwischen der Verzweigungsdichte und Länge von Capillaren im Neocortex der Ratte während der postnatalen Entwicklung. Z. Anat. Entwickl. Gesch. 141: 207–221 (1973).

Bär, Th.: Wirkung chronischer Hypoxie auf die postnatale Synaptogenese im Occipital-cortex der Ratte. Verh. Anat. Ges. 71: 915–924 (1977).

Bär, Th.: The vascular system of the cerebral cortex. Adv. Anat. Embryol. Cell. Biol., Vol. 59 (1980).

Bengoa, J.M.: The problem of malnutrition. WHO Cronicle 3: 3–12 (1974).

Benton, J.W., Moser, H.W., Dodge, P.R., Carr, S.: Modification of the schedule of myelination in the rat by early nutritional deprivation. Pediatrics 38: 801–807 (1966).

Berry, M., Rogers, A.W., Eayrs, J.T.: Pattern of cell migration during cortical histogenesis. Nature 203: 591–593 (1964).

Berry, M., Rogers, A.W.: The migration of neuroblasts in the developing cerebral cortex. J. Anat. (London) 99: 691–709 (1965).

Bliss, T.J.P., Chung, S.H.: An electrophysiological study of the hippocampus of the "reeler" mutant mouse. Nature 252: 153–155 (1974).

Bonavita, V.F., Ponte, F., Amore, G.: Lactate dehydrogenase enzymes in the nervous tissue. IV. An ontogenetic study on the rat brain. J. Neurochem. 11: 39–47 (1964).

Boulder committee: Embryonic vertebrate central nervous system: Revised terminology. Anat. Rec. 166: 257–262 (1970).

Bradbury, M.: The concept of a blood-brain barrier. Chichester, New York, Brisbane, Toronto, John Wiley & Sons, 1979.

Brizzee, K.R., Vogt, J., Karetchko, X.: Postnatal changes in glia/neuron index with a comparison of methods of cell enumeration in the white rat. Progr. Brain Res. 4: 136–146 (1963).

Brown, R.E.: Organ weight in malnutrition with special reference to brain weight. Dev. Med. Child. Neurol. 8: 512–522 (1966).

Burnham, P., Raiborn, C., Varon, S.: Replacement of nerve growth factor by ganglionic non-neuronal cells for the survival in vitro of dissociated ganglionic neurons. Proc. Natl. Acad. Sci. U.S.A. 69: 3556–3560 (1972).

Caley, D.M., Maxwell, D.S.: Development of the blood vessels and extra cellular spaces during postnatal maturation of rat cerebral cortex. J. Comp. Neurol. 138: 31–48 (1970).

Callison, D.A., Spencer, J.W.: Effect of chronic undernutrition and/or visual deprivation upon the evoked potential from the developing rat brain. Dev. Psychobiol. 1: 196–204 (1968).

Campbell, A.C.P.: Variation in vascularity and oxidase content in different regions of the brain of the cat. Arch. Neurol. Psychiat. (Chicago) 41: 223–242 (1937).

Caviness, V.S. Jr., Sidman, R.L.: Olfactory structures of the forebrain in the reeler mutant mouse. J. Comp. Neur. 145: 85–104 (1972).

Caviness, V.S. Jr., Sidman, R.L.: Time of origin of corresponding cell classes in the cerebral cortex of normal and reeler mutant mice. An autoradiographic analysis. J. Comp. Neur. 148: 141–152 (1973).

Chan-Palay, V., Palay, S., Wu, J.Y.: Gamma-aminobutyric acid pathways in the cerebellum studied by retrograde and anterograde transport of glutamic acid decarboxylase antibody after in vivo injections. Anat. Embryol. 157: 1–14 (1979).

Chase, H.P., Lindsey, W.F.B., O'Brien, D.: Undernutrition and cerebellar development. Nature, Lond. 221: 554–555 (1969).

Chase, H.P., Rodgerson, D.O., Lindsley, W. Jr., Thorne, T., Cheung, G.: Brain glucose utilization in undernourished rats. Pediat. Res. 10: 102–107 (1976).

Chen, S., Hillman, D.E.: Giant spines and enlarged synapses induced in Purkinje cells by malnutrition. Brain Res. 187: 487–493 (1980).

Chow, K.L., Leiman, A.L.: The structural and functional organization of the neo-cortex. Neurosci. Res. Program Bull. 8: 153–220 (1970).

Clos, J., Rabiere, A., Legrand, J.: Differential effects of hypothyroidism and undernutrition on the development of glial cells in the rat. Brain Res. 63: 445–449 (1973).

Clos, J., Favre, M., Schue-Matrat, M., Legrand, J.: Effects of undernutrition on cell formation in the rat brain and specially on cellular composition of the cerebellum. Brain Res. 123: 13–26 (1977).

Cobb, S., Talbott, J.H.: Studies in cerebral circulation. II. A quantitative study of cerebral capillaries. Trans. Assoc. Am. Physicians 42: 255–272 (1927).

Colmenares, A.G.: Effects of malnutrition on the development of the cerebral cortex of the rat. Soc. Neurosci. (6th Ann. Meeting), Toronto, Canada p. 210 (1976).

Conighi, R.: Studi sull' angiotectonica dell' encefalo. Rivista sperimentale de freniatria e medicum. 46: 411–427 (1922).

Cordero, M.E., Diaz, G., Araya, I.: Neocortex development during severe malnutrition in the rat. Am. J. Clin. Nutr. 29: 358–365 (1976).

Cragg, B.G.: The development of cortical synapses during starvation in the rat. Brain 95: 143–150 (1972).

Cragg, B.G.: The development of synapses in kitten visual cortex during visual deprivation. Exp. Neurol. 46: 445–451 (1975).

Craigie, E.H.: On the relative vascularity of various parts of the central nervous system of the albino rat. J. Comp. Neurol. 31: 429–464 (1920).

Craigie, E.H.: The vascularity of the cerebral cortex of the albino rat. J. Comp. Neurol. 33: 193–212 (1921).

Craigie, E.H.: Changes in the vascularity in the brain stem and cerebellum of the albino rat between birth and maturity. J. Comp. Neurol. 38: 27–48 (1924).

Craigie, E.H.: Postnatal changes in vascularity in the cerebral cortex of the male albino rat. J. Comp. Neurol. 39: 301–324 (1925).

Craigie, E.H.: The comparative anatomy and embryology of the capillary bed of the central nervous system. Proc. Assn. Res. Nerv. Ment. Dis. 18: 3–28 (1938).

Crnic, L.S.: Effects of infantile undernutrition on adult learning in rats. Methodological and design problems. Psychol. Bull. 83: 715–728 (1976).

Croskerry, P.G., Smith, G.K., Shepard, B.J., Freeman, K.B.: Perinatal brain DNA in the normal and growth hormone-treated rats. Brain Res. 52: 413–418 (1973).

Culley, W.J.: Brain energy reserves during early postnatal undernutrition. Fedr. Proc. 30: 459 (1971).

Dahl, D.R., Samson, F.E. Jr.: Metabolism of rat brain mitochondria during postnatal development. Amer. J. Physiol. 196: 470–472 (1959).

Dainat, J.: The incorporation in vivo of L–^{3}H-leucine into cerebellar proteins of the young normal, underfed, hyperthyroid and hypothyroid rat. J. Neurochem. 23: 713–719 (1974).

Das, G.D., Lammert, G.L., McAllister, J.P.: Contact guidance and migratory cells in the developing cerebellum. Brain Res. 69: 13–29 (1974).

Das, G.D.: Differentiation of Bergman glia cells in the cerebellum. A Golgi Study. Brain Res. 110: 199–213 (1976).

De Bault, L.E., Cancilla, P.A.: γ-glutamyl transpeptidase in isolated brain endothelial cells: Induction by glial cells in vitro. Science 207: 653–655 (1980).

DeLicardie, E.R., Cravioto, J.: Behavioral responsiveness of survivors of clinically severe malnutrition to cognitive demands. In: Early malnutrition and mental development. Symposia of the Swedish Nutrition Foundation XII (Cravioto, J., Hambraeus, L., Vahlquist, B. Eds.) Uppsala, Almquist & Wiksell 1974, pp. 134–153.

Delorme, P., Gayet, J., Grignon, G.: Ultrastructural study on transcapillary exchanges in the developing telencephalon of the chicken. Brain Res. 22: 269–283 (1970).

Deo, M.G.: Cell biology of Protein-Calorie Malnutrition. Wld. Rev. Nutr. Diet. Vol. 32, Basel, Karger 1978, pp. 49–95.

Desmond, M.E., Jacobson, A.G.: Embryonic brain enlargement requires cerebrospinal fluid pressure. Dev. Biol. 57: 188–198 (1977).

Devor, M., Caviness, V.S. Jr., Derer, P.: A normally laminated afferent projection to an abnormally laminated cortex: Some olfactory connections in the reeler mouse. J. Comp. Neur. 164: 471–482 (1975).
Dickerson, J.W.T., Pao, S.-K.: Effect of pre- and postnatal maternal protein deficiency on free amino acids and amines of rat brain. Biol. Neonate 25: 114–124 (1975).
Dobbing, J.: The influence of early nutrition on the development and myelination of the brain. Proc. R. Soc. Lond. (Biol.) 159: 503–509 (1964).
Dobbing, J., Sands, J.: Vulnerability of developing brain. IX. The effect of nutritional growth retardation on the timing of the brain growthspurt. Biol. Neonate 19: 363–378 (1971).
Dobbing, J., Hopewell, J.W., Lynch, A.: Vulnerability of developing brain. VII. Permanent deficits of neurons in cerebral and cerebellar cortex following early mild undernutrition. Exp. Neurol. 32: 439–447 (1971).
Donahue, S.: A relationship between fine structure and function of blood vessels in the central nervous system of rabbit fetuses. Am. J. Anat. 115: 17–26 (1964).
Duckett, S.: The establishment of internal vascularization in the human telencephalon. Acta Anat. 80: 107–113 (1971).
Dunning, H.S., Wolff, H.G.: The relative vascularity of various parts of the central and peripheral nervous system of the cat and its relation to function. J. Comp. Neurol. 67: 433–450 (1937).
Dyson, S., Jones, D.G.: Some effects of undernutrition on synaptic development. – A quantitative ultrastructural study. Brain Res. 114: 365–378 (1976a).
Dyson, S.E., Jones, D.G.: Undernutrition and the developing nervous system. Progr. Neurobiol. 7: 171–196 (1976b).
Eayrs, J.T., Horn, G.: The development of cerebral cortex in hypothyroid and starved rats. Anat. Rec. 121: 53–61 (1955).
Eayrs, J.T.: The vascularity of the cerebral cortex in normal and cretinous rats. J. Anat. 88: 164–173 (1954).
Eins, S., Wilhelms, E.: Assessment of preparative volume changes in central nervous tissue using automatic image analysis. Microscope 24: 29–38 (1976).
Eins, S., Bär, Th.: Orientation distribution of blood vessels in central nervous tissue. Prakt. Metallogr. 8: 381–388 (1978).
Elias, H., Henning, A., Schwartz, D.E.: Stereology: Applications to biomedical research. Physiol. Rev. 51: 158–200 (1971).
Engsner, G., Belate, S., Sjögren, I., Vahlquist, B.: Brain growth in children with marasmus. Ups. J. Med. Sci. 79: 116–128 (1979a).
Engsner, G., Hobte, D., Sjögren, I., Vahlquist, B.: Brain growth in children with kwashiorkor. Acta Pediatr. Scand. 63: 687–694 (1974b).
Farkas-Bargeton, E., Diebler, M.F.: A topographical study of enzyme maturation in human cerebral neocortex: A histochemical and biochemical study. In: Architectonics of the Cerebral Cortex. (Brazier, M.A.B. and Petsche, H. Eds.) New York, Raven Press 1978, pp. 175–190.
Fazzari, I.: La circolazione arteriosa della corteccia cerebellare. Studio comparative. Rivista di Patologia nervosa e mentale 29: 425–459 (1924).
Feeney, J.F. Jr., Watterson, R.L.: The development of the vascular pattern within the walls of the central nervous system of the chick embryo. J. Morphol. 78: 231–304 (1946).
Fish, I., Winick, M.: Cellular growth in various regions of the developing rat brain. Expl. Neurol. 25: 313–328 (1969a).

Fish, I., Winick, M.: Effect of malnutrition on regional growth of the developing rat brain. Exp. Neurol. 25: 534–540 (1969b).

Friede, R.L.: Topographic Brain Chemistry. New York, London, Academic Press 1966.

Fujita, S.: The matrix cell and cytogenesis in the developing central nervous system. J. Comp. Neurol. 120: 37–42 (1963).

Gambetti, P., Autilio-Gambetti, L., Gonatas, N.K., Schafer, B., Stieber, A.: Synapses and malnutrition: Morphological and biochemical study of synaptosomal fraction from rat cerebral cortex. Brain Res. 47: 477–478 (1972).

Gambetti, P., Autilio-Gambetti, L., Rizzuto, N., Schafer, B., Pfaff, L.: Synapses and malnutrition: Quantitative ultrastructural study of rat cerebral cortex. Expl. Neurol. 43: 464–473 (1974).

Globus, A., Scheibel, A.B.: The effect of visual deprivation on cortical neurons. A Golgi study. Exp. Neurol. 19: 331–345 (1967).

Gourdon, J., Clos, J., Coste, C., Dainat, J., Legrand, J.: Comparative effects of hypothyroidism, hyperthyroidism and undernutrition on the protein and nucleic acid content of the cerebellum in the young rat. J. Neurochem. 21: 861–871 (1973).

De Guglielmone, A.E.R., Soto, A.M., Duvilanski, B.H.: Neonatal undernutrition and RNA synthesis in developing rat brain. J. Neurochem. 22: 529–533 (1974).

Gyllensten, L.: Postnatal development of the visual cortex in darkness (mice). Acta Morphol. Neerl.-Scand. 2: 331–345 (1959a).

Gyllensten, L.: Influence of oxygen exposure on the postnatal vascularization of the cerebral cortex in mice. Acta Morphol. Neerl.-Scand. 2: 289–310 (1959b).

Güldner, F.-H., Wolff, J.R.: Perisynaptic reactions of astroglia in the visual cortex after optic nerve stimulation. Exp. Brain Res., Suppl. 1: 343–346 (1976).

Haddara, M.A., Nooreddin, M.A.: A quantitative study on the postnatal development of the cerebellar vermis of mouse. J. Comp. Neur. 128: 245–254 (1966).

Haltia, M.: Postnatal development of spinal anterior horn neurones in normal and undernourished rats. A quantitative cytochemical study. Acta Physiol. Scand., Suppl. 352 (1970).

Hamberger, A., Sourander, P.: The influence of early protein-calorie malnutrition on neuronal and glial protein synthesis. An experimental study on rats. Neurochem. Res. 3: 535–547 (1978).

Hinds, J.W., Ruffet, T.L.: Cell proliferation in the neural tube: An electron microscopic and Golgi analysis in the mouse cerebral vesicle. Z. Zellforsch. 115: 226–264 (1971).

Hökfelt, T., Ljungdahl, A.: Cellular localization of labeled gamma-amino butyric acid (^{3}H–GABA) in rat cerebellar cortex: an autoradiographic study. Brain Res. 22: 391–396 (1970).

Hökfelt, T., Ljungdahl, A.: Autoradiographic identification of cerebral and cerebellar cortical neurons accumulating labeled gamma-amino-butyric acid (^{3}H–GABA). Exp. Brain Res. 14: 354–362 (1972).

Im, H.S., Barnes, R.H., Levitsky, D.A.: Postnatal malnutrition and brain cholinesterase in rats. Nature 233: 269–270 (1971).

Jacobson, M.: Developmental Neurobiology, 2nd Ed., New York and London, Plenum Press 1978.

Kaplan, H.A., Ford, D.H.: The Brain Vascular System. Amsterdam, Elsevier 1966.

Katiyar, G.P., Agarwal, K.N., Shanker, R., Nagchaudhuri, J.: Effect of protein energy deprivation on the brain enzymes of glutamic acid in preweaning rats. Nutr. Metab. 20: 396–403 (1976).

Källén, B.: Early morphogenesis and pattern formation in the central nervous system. In: Organogenesis. (DeHaan, R.L. & Ursprung, H. Eds.) New York, Holt, Reinhart and Winston 1965, pp. 107–128.

Kissane, J.Q., Hawrylewicz, E.J.: Development of N^+/K^+ ATPase in neonatal rat brain synaptosomes after protein malnutrition. Ped. Res. 9: 46–150 (1975).
Klossovskii, B.N.: The development of the brain. New York Pergamon Press 1963.
Koshy, T.S., Sara, V.R., King, T.L., Lazarus, L.: The influence of protein restriction imposed at various stages of pregnancy on fetal and placental development. Growth 39: 497–506 (1975).
Krieg, W.J.S.: Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. J. Comp. Neurol. 84: 221–275 (1946).
Kuckuk, B.: Über die Entwicklung und Chemodifferenzierung des Kleinhirns der Ratte. Histochemie 9: 217–255 (1967).
Lange, W., Halata, Z.: Comparative studies on the pre- and postterminal blood vessels in the cerebellar cortex of rhesus monkey, cat and rat. Anat. Embryol. 158: 51–62 (1979).
Larsell, O.: The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. J. Comp. Neurol. 97: 281–357 (1952).
Latham, M.C.: Protein-calorie malnutrition in children and its relation to psychological development and behavior. Physiol. Rev. 54: 541–565 (1974).
Legrand, J.: Morphologic and biochemical effects of hormones on the developing nervous system in mammals. In: Brain, Fetal and Infant. (Berenberg, S.R. Ed.) The Hague, M. Nijhoff 1977, pp. 137–164.
Levi-Montalcini, R.: Growth and differentiation in the nervous system. In: The nature of biological diversity (Allen, J.M. Ed.) New York, McGraw-Hill 1963, pp. 261–295.
Lewis, O.J.: The form and development of the blood vessels of the mammalian cerebral cortex. J. Anat. (Lond.) 91: 40–46 (1957).
Lewis, P.D.: Cell death in the germinative layer of the postnatal rat brain. Neuropath. appl. Neurobiol. 1: 21–29 (1975).
Lewis, P.D., Balazs, R., Patel, A.J., Johnson, A.L.: The effect of undernutrition in early life on cell-generation cycle in the brain. Brain Res. 83: 235–247 (1975).
Lierse, W.: Uber die Beeinflussung der Hirnangioarchitektur durch die Morphogenese. Acta Anat. 53: 1–54 (1963).
McCarthy, K.D., Partlow, L.M.: Neuronal stimulation of (^{3}H)-thymidine incorporation by primary culture of highly purified non-neuronal cells. Brain Res. 114: 415–426 (1976).
McConell, P., Berry, M.: The effect of refeeding after neonatal starvation on the Purkinje cell dendritic growth in the rat. J. Comp. Neur. 178: 759–772 (1978).
McCoy, R.H.: Dietary requirements of the rat. In: The rat in laboratory investigation. (Farris, E.J. & Griffith, J.Q. Eds.) New York, Hafner 1963, pp. 68–103.
McKhann, G.M., Albers, R.W., Sokoloff, L., Michelsen, O., Tower, D.B.: The quantitative significance of the gamma-amino butyric acid pathway in cerebral oxidative metabolism. In: Inhibition in the nervous system and gamma-amino butyric acid. New York, Pergamon 1960, pp. 169–181.
Malmgren, L., Olsson, Y.: A sensitive histochemical method for light- and electronmicroscopic demonstration of horse-radish peroxidase. J. Histochem. Cytochem. 25: 1280–1283 (1977).
Mares, V., Lodin, Z.: The cellular kinetics of the developing mouse cerebellum. II. The function of the external granular layer in the process of gyrification. Brain Res. 23: 343–352 (1970).
Marin-Padilla, M.: Prenatal and early postnatal ontogenesis of the human motor cortex: A Golgi study. I. The sequential development of the cortical layers. Brain Res. 23: 167–183 (1970).
Martin, A.H., Langman, J.: The development of the spinal cord examined by autoradiography. J. Embryol. Exp. Morphol. 14: 23–35 (1965).

Martin, A.H.: Significance of mitotic spindle fibre orientation in the neural tube. Nature 216: 1133–1134 (1967).

Massaro, T.F., Levitsky, D.A., Barnes, R.H.: Protein malnutrition in the rat: Its effects on maternal behavior and pup development. Dev. Psychobiol. 7: 551–561 (1974).

Milstein, J.M., Swaiman, K.F.: Oxidative phosphorylation in developing rat brain. Neurology 17: 302 (1967).

Minchin, M.C.W., Iversen, L.L.: Release of (^{3}H)-gamma-amino butyric acid from glial cells in the rat dorsal root ganglia. J. Neurochem. 23: 533–540 (1974).

Moore, B.W., Menke, R., Prensky, A.L., Fishman, M.A., Agrawal, H.C.: Selective decreases of S–100 in discrete anatomical areas of undernourished rat brain. Neurochem. Res. 2: 549–553 (1977).

Morest, D.K.: The growth of dendrites in the mammalian brain. Z. Anat. Entw. Gesch. 128: 290–317 (1969).

Morgane, P.J., Miller, M., Kemper, T., Stern, W., Forbes, W., Hall, R., Bronzino, J., Kissane, J., Hawrylewicz, E., Resnick, O.: The effects of protein malnutrition on the developing central nervous system in the rat. Neurosci. Biobehavior. Res. 137–230 (1978).

Mountcastle, V.B.: Modality and topographic properties of single neurons of cat's somatic sensory cortex. J. Neurophysiol. 20: 408–434 (1957).

Mourek, J., Himwich, W.A., Myslivecek, J., Callison, D.: The role of nutrition in the development of evoked cortical responses in rat. Brain Res. 6: 241–251 (1967).

Muzzo, S., Gregory, T., Gardner, L.T.: Oxygen consumption by brain mitochondria of rats malnourished *in utero*. J. Nutr. 103: 314–317 (1973).

Neville, H.E., Chase, H.P.: Undernutrition and cerebellar development. Exp. Neurol. 33: 485–497 (1971).

Nowak, T.S., Munro, H.N.: Effects of protein-calorie malnutrition on biochemical aspects of brain development. In: Nutrition and the Brain, Vol. 2 (Wurtman, R.J. and Wurtman, J.J. Eds.) New York, Raven Press 1977, pp. 193–260.

Oldfors, A., Sourander, P.: The effect of undernutrition on spinal ganglion nerve cell size. Acta Neuropath. (Berl.) 89: 75–80 (1977).

Oldfors, A., Ullman, M.: Motor nerve conduction velocity and nerve fibre diameter in experimental protein deprivation. Studies on rat peripheral nerve during development. Acta Neuropath. (Berl.) 1980 (in press).

Palay, S.: The morphology of synapses in the central nervous system. Exp. Cell Res. (Suppl.) 5: 275–293 (1958).

Patel, A.J., Balazs, R.: Manifestation of metabolic compartmentation during the maturation of the rat brain. J. Neurochem. 17: 955–971 (1970).

Patel, A.J., Balazs, R., Johnsson, A.L.: Effect of undernutrition on cell formation in the rat brain. J. Neurochem. 20: 1151–1165 (1973).

Patel, A.J., Atkinson, D.J., Balazs, R.: Effect of undernutrition on metabolic compartmentation of glutamate and on the incorporation of ^{14}C-leucine into protein in developing rat brain. Dev. Psychobiol. 8: 453–464 (1975).

Persson, L., Sima, A.: The effect of pre- and postnatal undernutrition on the development of the cerebellar cortex in the rat. II. Histochemical observations. Neurobiology 5: 151–166 (1975).

Pfaffenroth, M.J., Das, M.J.: Heterotopic cell nests in the developing rat cerebellum. Acta Neuropath. (Berl.) 30: 1–9 (1974).

Pfeifer, R.A.: Die Angioarchitektonik der Grosshirnrinde. Berlin, Springer 1928.
Plant, S.M.: Studies of undernutrition in the young rat: Methodological considerations. Dev. Psychobiol. 3: 157–167 (1970).
Punch, J., Ivinskis, A.: Protein deprivation and behavior: Conceptual and methodological problems. Behav. biol. 19: 108–129 (1977).
Pysh, J.J., Perkins, R.E.: Undernutrition and Purkinje cell development. Neurosci. Abstr. 1: 756 (1975).
Raedler, A., Sievers, J.: Light and electronmicroscopical studies on specific cells of the marginal zone in the developing rat cerebral cortex. Anat. Embryol. 149: 173–181 (1976).
Rajalakshmi, R., Pillai, K.R., Ramakrishnan, C.V.: Effects of different supplements to low protein and poor quality protein diets on performance and brain enzymes in the albino rat. J. Neurochem. 16: 599–606 (1969).
Rajalakshmi, R., Parameswaran, M., Ramakrishnan, C.V.: Effects of different levels of dietary protein on brain glutamate dehydrogenase and decarboxylase in albino rats. J. Neurochem. 23: 123–127 (1974).
Rakic, P.: Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in Macacus Rhesus. J. Comp. Neur. 141: 283–312 (1971).
Rakic, P.: Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. 145: 61–84 (1972).
Rakic, P.: Neurons in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. Science 183: 425–427 (1974).
Richardsson, K.C., Jarrett, L., Finke, E.H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35: 313–323 (1960).
Rickmann, M., Wolff, J.R.: On the earliest stages of glial differentiation in the neocortex of rat. Exp. Brain Res., Suppl. I, 239–243 (1976).
Rickmann, M., Chronwall, B.M., Wolff, J.R.: On the development of nonpyramidal neurons and axons outside the cortical plate. The early marginal zone as a pallial anlage. Anat. Embryol. 151: 285–307 (1977).
Roach, M.K., Corbin, J., Pennington, W.: Effect of undernutrition on amino acid compartmentation in the developing rat brain. J. Neurochem. 22: 521–528 (1974).
Robain, D., Ponsot, G.: Effects of undernutrition on glial maturation. Brain Res. 149: 379–397 (1978).
Salas, M., Cintra, L.: Nutritional influences upon somato sensory evoked responses during development in the rat. Physiol. Behav. 10: 1019–1022 (1973).
Salas, M., Diaz, S., Nieto, A.: Effects of neonatal food deprivation on cortical spines and dendritic development in the rat. Brain Res. 73: 139–144 (1974).
Samson, F.E. Jr., Balfour, W.M., Jacobs, R.J.: Mitochondrial changes in developing rat brain. Am. J. Physiol. 199: 693–696 (1960).
Sauer, F.C.: Mitosis in the neural tube. J. Comp. Neurol. 62: 377–405 (1935a).
Sauer, F.C.: The cellular structure of the neural tube. J. Comp. Neurol. 63: 13–23 (1935b).
Sauer, F.C.: The interkinetic migration of embryonic epithelial nuclei. J. Morphol. 60: 1–11 (1936).
Sauer, M.E., Chittenden, A.C.: Deoxyribonucleic acid content of cell nuclei in the neural tube of the chick embryo: Evidence for intermitotic migration of nuclei. Exp. Cell. Res. 16: 1–6 (1959).
Sauer, M.E., Walker, B.E.: Radiographic study of interkinetic nuclear migration in the neural tube. Proc. Soc. Exp. Biol. Med. 101: 557–560 (1959).

Saunders, R.L. de C.H., Bell, M.A.: X-ray microscopy and histochemistry of the human cerebral blood vessels. J. Neurosurg. 35: 128–140 (1971).
Saxén, L., Toivonen, S.: Primary Embryonic Induction. London, Logos 1962.
Schadé, J.P., Meeter, K., Groeningen, W.B. van: Maturational aspects of the dendrites in the human cerebral cortex. Acta Morphol. Neerl.-Scand. 5: 37–48 (1962).
Scharrer, E.: The functional significance of the capillary bed in the brain of the opossum. Anat. Rec. 75: 319–340 (1939).
Scharrer, E.: The blood vessels of the nervous tissue. Quart. Rev. Biol. 19: 308–318 (1944).
Scharrer, E.: Capillaries and mitochondria in neuropil. J. Comp. Neur. 83: 237–243 (1945).
Schmechel, D.E., Rakic, P.: A Golgi study of radial glial cells in developing monkey telencephalon: Morphogenesis and transformation into astrocytes. Anat. Embryol. 156: 115–152 (1979).
Scrimshaw, N.S., Taylor, C.E., Gordon, J.E.: Interactions of nutrition and infection. World Health Organization Monograph Series No. 57, Geneva, World Health Organization (1968).
Seil, F.Z., Kelly, J.M., Leiman, A.L.: Anatomical organization of cerebral neocortex in tissue culture. Exp. Neurol. 45: 435–450 (1974).
Seiler, N.: Enzymes. In: Handbook of Neurochemistry. (Lajtha, A. Ed.) New York, Plenum 1969, pp. 325–468.
Sellström, A., Hamberger, A.: Potassium-stimulated γ-amino butyric acid release from neurons and glia. Brain Res. 119: 189–198 (1977).
Sereni, F., Principi, N., Perletti, L., Piceni-Sereni, L.: Undernutrition and the developing rat brain. I. Influences on acetylcholinesterase and succinic acid dehydrogenase activities and on norepinephrine and 5–OH tryptamine tissue concentrations. Biol. Neonate 10: 254–265 (1966).
Shoemaker, W.J., Bloom, F.E.: Effect of undernutrition on brain morphology. In: Nutrition and the brain, Vol. 2. (Wurtman, R.J., Wurtman, J.J. Eds.) New York, Raven Press 1977, pp. 147–192.
Shrader, R.E., Zeman, F.J.: Effect of maternal protein deprivation on morphological and enzymatic development of neonatal rat tissue. J. Nutr. 99: 401–412 (1969).
Siassi, F., Siassi, B.: Differential effects of protein-calorie restriction and subsequent repletion on neuronal and non-neuronal components of cerebral cortex in newborn rats. J. Nutr. 103: 1625–1633 (1973).
Sidman, R.L., Miale, I.L., Feder, N.: Cell proliferation and migration in the primitive ependymal zone: An autoradiographic study of histogenesis in the nervous system. Exp. Neurol. 1: 322–333 (1959).
Sima, A.: Relation between the number of myelin lamellae and axon circumference in fibres of ventral and dorsal roots and optic nerve in newborn, undernourished and rehabilitated rats. Acta Physiol. Scand. Suppl. 410: 1–27 (1974).
Sima, A., Persson, L.: The effect of pre- and postnatal undernutrition on the development of the rat cerebellar cortex. I. Morphological observations. Neurobiology 5: 23–34 (1975).
Sjöstrand, J.: Proliferative changes in glial cells during nerve regeneration. Z. Zellforsch. 68: 481–493 (1965).
Sjöstrand, J.: Morphological changes in glial cells during nerve regeneration. Acta Physiol. Scand. Suppl. 270: 19–43 (1966).
Sobotka, T.J., Cook, M.P., Bradie, R.E.: Neonatal malnutrition: Neurochemical, hormonal and behavioral manifestations. Brain Res. 65: 443–457 (1974).
Sotelo, C.: Purkinje cell ontogeny: Formation and maintenance of spines. Progr. Brain Res. 48: 149–170 (1970).

Sourander, P., Sima, A., Haltia, M.: Malnutrition and morphological development of the nervous system. Jfr. In: Early malnutrition and mental development. Symposia of the Swedish Nutrition Foundation XII (Cravioto, J., Hambraeus, L., Vahlquist, B. Eds.) Uppsala, Almquist & Wiksell 1974, pp. 39–54.

Sourander, P., Sima, A., Schucht, H.: Effect of pre- and postnatal protein-calorie undernutrition on RNA-content in rat motor neurons. Acta Neurol. Scandinav. 53: 209–216 (1976).

Stewart, R.J.C., Merat, A., Dickerson, J.W.T.: Effect of a low protein diet in mother rats on the structure of the brains of the offspring. Biol. Neonate 25: 125–134 (1974).

Strong, L.H.: The early embryonic pattern of internal vascularization of mammalian cerebral cortex. J. Comp. Neurol. 123: 121–138 (1964).

Sugita, N.: Comparative studies on the growth of the cerebral cortex VII. On the influence of starvation at an early age upon the development of the cerebral cortex. Albino rat. J. Comp. Neurol. 29: 177–240 (1918).

Swaiman, K.F.: Energy and electrolyte changes during maturation. In: Developmental Neurobiology. (Himwich, W.A. Ed.) Springfield, Thomas Books 1970, pp. 311–330.

Swaiman, K.F., Wolfe, R.N.: The effect of food deprivation and refeeding on lactic dehydrogenase activity in immature rat brain. Proc. Soc. Exp. Biol. Med. 134: 185–187 (1970).

Tilney, F., Casamajor, L.: The development of hemal channels in the central nervous system of the albino rat. Anat. Rec. 11: 425–428 (1917).

Uchimura, Y.: Uber die Blutversorgung der Kleinhirnrinde und ihre Bedeutung für die Pathologie des Kleinhirns. Z. Gesamte Neurol. Psychiat. 120: 774–782 (1929).

Valverde, F.: Apical dendritic spines of the visual cortex and light deprivation in the mouse. Exp. Brain Res. 3: 337–352 (1967).

Valverde, F.: Structural changes in the area striata of the mouse after enucleation. Exp. Brain Res. 5: 274–292 (1968).

Varon, S., Raiborn, C., Norr, S.: Association of antibody to nerve growth factor with ganglionic nonneurons (glia) and consequent interference with their neuron-supportive action. Exp. Cell. Res. 88: 247–256 (1974).

Viteri, F., Béhar, M., Arroyave, G., Scrimshaw, N.S.: Clinical aspects of protein malnutrition. In: Mammalian protein metabolism. Vol II. (Munro, H.N., Allison, J.B. Eds.) New York and London, Academic Press 1964, pp. 523–568.

West, M.J., del Cerro, M.: Early formation of synapses in the molecular layer of the fetal rat cerebellum. J. Comp. Neurol. 165: 137–160 (1976).

West, C.D., Kemper, T.L.: The effect of a low protein diet on the anatomical development of the rat brain. Brain Res. 107: 221–237 (1976).

Williams, R.G.: The development of vascularity in the hindbrain of the chick. J. Comp. Neurol. 66: 77–101 (1937).

Winick, M., Noble, A.: Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Devl. Biol. 12: 451–466 (1965).

Winick, M.: Malnutrition and brain development. J. Pediat. 74: 667–679 (1969).

Winick, M.: Cellular growth in intrauterine malnutrition. Pediatr. Clin. North. Am. 17: 69–78 (1970a).

Winick, M.: Nutrition and nerve cell growth. Fed. Proc. 29: 1510–1515 (1970b).

Wislocki, G.B., Campbell, A.C.P.: The unusual manner of vascularization of the brain of the opossum (Didelphys. virginiana). Anat. Rec. 67: 177–191 (1937).

Wolff, J.R.: Ein Beitrag zur Ultrastruktur der Blutkapillaren: Das nahtlose Endothel. Z. Zellforsch. 64: 290–300 (1964).
Wolff, J.R.: The astrocyte as link between capillary and nerve cell. Triangle 9: 153–164 (1970).
Wolff, J.R., Bär, Th.: Seamless endothelia in brain capillaries during development of the rat's cerebral cortex. Brain Res. 41: 17–24 (1972).
Wolff, J.R., Goerz, Ch., Bär, Th., Güldner, F.H.: Common morphogenetic aspects of various organotypic microvascular patterns. Microvascular Res. 10: 373–395 (1975).
Wolff, J.R., Bär, Th.: Development and adult variations of the pericapillary glial sheath in the cortex of cat. In: The Cerebral Vessel Wall. (Cervós-Navarro, J. Ed.) New York, Raven Press 1976, pp. 7–13.
Wolff, J.R.: Ontogenetic aspects of cortical architecture: Lamination. In: Architectonics of the cerebral cortex. (Brazier, M.A.B., Petsche, H. Eds.) New York, Raven Press 1978, pp. 159–173.
Wolff, J.R., Chronwall, B.M., Rickmann, M.: Morphogenetic relations between cell migration and synaptogenesis in the neo-cortex of the rat. In: Proc. Europ. Soc. Neurochem., Vol. I (Neuhoff, V. Ed.) Weinheim, Verlag Chemie 1978, pp. 158–173.
Wolff, J.R., Rickmann, M., Chronwall, B.M.: Axo-glial synapses and GABA-accumulating glial cells in the embryonic neo-cortex of the rat. Cell. Tiss. Res. 201: 239–248 (1979).
Wolff, J.R.: Some morphogenetic aspects of the CNS development. In: Behavioral Development. The Bielefeld Interdisciplinary Project. New York, Cambridge University Press (1979a) (in press).
Wolff, J.R.: Hinweise auf eine Doppelrolle von GABA als synaptischer Transmitter und als morphogenetischer Faktor. Deutsche Zoologischen Gesellschaft (1979b) (im Druck).
Zahlava, J., Myslivecek, J.: Functional ability of cerebral cortex estimated by evoked potentials (EP) in normal and starved puppies. Activitas nerv. sup. 10: 209–210 (1968).
Zamenhof, S., Marthens, E. van, Margolis, F.L.: DNA (cell number) and protein in neonatal brain. Alterations by maternal dietary protein restriction. Science 160: 322–323 (1968).
Zamenhof, S., Marthens, E. van, Grauel, L.: DNA (cell number) and protein in neonatal rat brain: Alteration by timing of maternal protein restriction. J. Nutr. 101: 1265–1270 (1971).
Zamenhof, S., Marthens, E. van: Study of factors influencing prenatal brain development. Mol. Cell. Biochem. 4: 157–168 (1974).
Zeman, F.J., Stanbrough, E.C.: Effect of maternal protein deficiency on cellular development in the fetal rat. J. Nutr. 99: 274–282 (1969).

Printed in Sweden. Gotab, Kungälv. 1980.29280